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Studies on the Single Injection Technique
for Determination of Renal Clearance

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From the Department of Surgery and the Isotope Laboratory
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STUDIES ON THE SINGLE INJECTION TECHNIQUE
FOR DETERMINATION OF RENAL CLEARANCE

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Pihl, B. & Nosslin, B. The single injection technique for determination of renal clearance I. Clearance of iothalamate and iodohippurate in dogs.

Pihl, B. The single injection technique for determination of renal clearance II. Experimental studies on methodological problems.

Pihl, B. The single injection technique for determination of renal clearance III. Renal extraction of iothalamate, iodohippurate and Cr-EDTA.

Nosslin, B. & Pihl, B. The single injection technique for determination of renal clearance IV. Clearance calculations and kinetics of the test substances.

Pihl, B. The single injection technique for determination of renal clearance V. A comparison with the continuous infusion technique in the dog and in man.

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INTRODUCTION

In 1929 Möller, McIntosh and Van Slyke introduced the term 'clearance' into renal physiology. It was defined as the volume of blood that one minute's excretion of urine suffices to clear of urea. Since then the term has come into widespread use to describe the excretory activity of the kidneys for different substances. The classical method for clearance determination requires a continuous infusion of the test substance to keep the plasma level constant, and urine collection in several periods (Smith 1951, 1956). In 1940, however, Alving and Miller described a method for measurement of clearance after a single injection of the test substance, but still requiring urine collection. In the same year Barnett described a way of estimating the clearance after a single injection of the test substance and without urine collection. This method was developed into a real clearance measurement by Newman, Bordley & Winternitz in 1944. The so called single injection method for determination of renal clearance, with or without urine collection, has since then been used by many investigators, but opinions as to its value are diverging and the reports in the literature are contradictory. Since the differences of opinion largely seem to be due to variations in performance of the test and in calculation of the clearance values, it was decided to reinvestigate the single intravenous injection clearance and its relation to the continuous infusion clearance, using methods recently devised by Nosslin (1965). Main emphasis was laid on the renal clearance proper, as determined from the plasma curve and the urinary excretion, but the plasma values were also used for estimation of the total clearance.

MATERIAL, METHODS AND RESULTS

I. Clearance of iothalamate and iodohippurate in dogs

Renal clearance of iothalamate and iodohippurate labelled with radioactive iodine was investigated in three series of dogs, using the single injection technique. The clearance values were calculated from the amount excreted in the urine and the area under the venous plasma curve during the corresponding time period as described by Nosslin (1965). In each experiment four separate determinations from consecutive periods following the injection were performed. Comparison was made with simultaneous continuous infusion clearance of the same substance.

For iothalamate the single injection clearance agreed very well with the continuous infusion clearance, the mean values being 3.3 ml/min/kg and 3.5 ml/min/kg, respectively. Individual determinations showed a high degree of correlation between the two techniques ($r + 0.98$). After single injection of iodohippurate a gradual fall of the clearance values from period to period was found. The mean values for the first period in the two series investigated were 9.86 and 10.20 ml/min/kg, respectively. and the fourth period values were 5.74 and 6.69 ml/min/kg, respectively. The values of the fourth period were thus only about 60 % of the first period values. The continuous infusion clearance of iodohippurate also showed a decrease which, however, was less pronounced, the first period value being 9.12 and the last period value 7.95 ml/min/kg.

The total clearance calculated from the plasma curve was for iothalamate 10 % higher and for iodohippurate 20 % higher than the continuous infusion clearance.

It is concluded that the clearance of iothalamate is constant during falling plasma concentration, and that it can be determined equally well with the single injection and the continuous infusion technique. The clearance of iodohippurate on the other hand is not constant under these conditions and the two techniques do not give comparable results.

II. Experimental studies on methodological problems

Several factors that might influence the single injection clearance values of radioactive iodohippurate, iothalamate and Cr-EDTA were studied in dogs and in man.

The influence of free iodine was studied in two series of dogs, and each per cent free iodine in iodohippurate was found to cause a decrease of the clearance values with 0.4 ml/min/kg during continuous infusion. When single injection was used no influence could be demonstrated.

The influence of carrier was investigated in paired experiments in dogs (with and without carrier), and in a few single investigations in patients. The addition of carrier to the test substance had no demonstrable influence on the clearance values, speaking against significant protein-binding.

The uptake of the test substances into the red blood cells was studied in vitro and in vivo in the dog as well as in man. No uptake into the red cells

was found for iothalamate or Cr-EDTA. The in vitro studies showed 26 % of the iodohippurate to be in the cells of dog blood and 12 % in human blood cells at equilibrium. The same distribution was found during continuous infusion in vivo, while after single injection 35 % and 24 % of the activity was in the red cells of dog blood and human blood, respectively. The immediate centrifugation of the blood samples during an iodohippurate clearance test was found important because of a secondary shift of the test substance between cells and plasma in vitro.

Resorption from the urinary bladder and recirculation of the test substance was found insignificant in investigations on two dogs.

The difference in concentration of the test substance between arterial and venous blood was studied after single injection and during continuous infusion in dogs and in man. After single injection the V/A-quotient always started below 1.0 but continuously increased to reach about 1.4 in the dog and 1.3 in man for iodohippurate, corresponding figures for iothalamate being 1.1 and 1.08. Clearance determinations performed on venous blood therefore resulted in progressively decreasing clearance values.

The urinary tract delay was investigated in dogs by clearance determinations with frequent and short urinary collection periods. The delay time was estimated from the dislocation in time between the clearance curve and the excretion curve and was found to be two minutes.

From the investigations it is concluded that two of the factors studied, namely the presence of free iodine in the test substance and the arterio-venous difference, contribute to the decrease in the single injection clearance values.

III. Renal extraction of iothalamate, iodohippurate and Cr-EDTA

The renal extraction of iothalamate, iodohippurate and Cr-EDTA was investigated by percutaneous renal vein catheterization in dogs and in man. The renal extraction of iothalamate in dogs was constant after single injection as well as during continuous infusion and amounted to 22 - 23 %. The correlation between the values obtained by the two techniques was excellent ($r + 0.98$). The extraction of Cr-EDTA after single injection in dogs was not significantly different from that of iothalamate. The investigations in patients showed the renal extraction of Cr-EDTA given as a single injection and of iothalamate given as a continuous in-

fusion to be constant with time, mean values being 17 % for both. Iodohippurate given by continuous infusion showed an essentially constant extraction in the dog as well as in patients, mean values being 75 % and 80 %, respectively. After single injection of iodohippurate the extraction decreased progressively with time. In the dogs the extraction in plasma started at 87 % and had diminished to 62 % after two hours. In the patients the corresponding figures were 89 % and 60 %.

The addition of carrier to the test substance had no influence on the renal extraction in the case of iothalamate but caused a somewhat lower extraction in the case of iodohippurate.

The influence of red cell uptake on the extraction was investigated by calculation of theoretical extraction values, which were found to be very close to the observed values, speaking against any such influence.

In the dogs a decrease of the clearance values corresponding to the decrease in the extraction values was found.

The conclusion is that the decrease in the renal extraction of iodohippurate after single injection explains a substantial part of the fall in the clearance values earlier found.

IV. Clearance calculations and kinetics of the test substances

Different methods for calculation of clearance and distribution volume were applied to plasma disappearance curves and urinary excretion curves obtained after single injection of iothalamate and iodohippurate in the dog and in man. The methods exhibited significant differences, and the deviations from the correct value were greater the simpler the model. This was especially the case for iodohippurate. When the calculations were performed on incompletely followed curves the clearance was in general overestimated while the distribution volume was underestimated.

An analysis of the influence of the free iodine contained in iodohippurate preparations showed that a significant decrease of the resulting clearance and extraction rate could be expected after single injection. The decrease caused by the 0.7 % iodine in the iodohippurate used should amount to 10 %. Since a much greater decrease has been found a further mechanism is proposed, consisting of a partial backflow into the blood of iodohippurate taken up by the kidney. Theoretical models are proposed for the

kinetics of the test substances, including estimates of the values for the rate constants and pool sizes.

It is concluded that although methods for a theoretically correct calculation of clearance and distribution volume are now available practical problems may still in certain situations limit the usefulness of single injection methods.

V. A comparison with the continuous infusion technique in the dog and in man

The clearance of radioactive iothalamate and iodohippurate was investigated in dogs and in man using concomitant single injection and continuous infusion of the test substance.

For iothalamate the renal single injection clearance, calculated on arterial plasma or blood values was equal to the continuous infusion clearance, the mean values being 2.9 ml/min/kg in the dog and 96 ml/min/1.73 m² in man. In the patients also peripheral venous blood was obtained and clearance calculated on these values showed a slight decrease with time amounting to 1.6 % per period. The total plasma clearance was 17 % higher than the continuous infusion clearance in the dog and 5 - 7 % higher in man. The different methods for iothalamate clearance showed a high degree of correlation in the dog as well as in man ($r > 0.95$).

For iodohippurate the continuous infusion clearance values were constant in the dog but somewhat decreasing in man. The arterial single injection clearance values decreased with 3.9 % per period in the dog and with 6.5 % per period in man, where the decrease of the venous single injection clearance was even more pronounced (8.9 % per period). The total plasma clearance as calculated on arterial plasma was in the dog 22 % higher than, but in man equal to, the continuous infusion clearance. All changes were more pronounced when the calculations were performed on whole blood values. The correlations between the different iodohippurate clearances were excellent in the dog as well as in man ($r > 0.95$).

It is concluded that for iothalamate the single injection technique, from a practical clinical point of view, gives the same clearance values as the continuous infusion technique, while for iodohippurate the single injection method and the continuous infusion method do not give the same results.

GENERAL DISCUSSION

The main difference between the continuous infusion method and the single injection method is the steady state achieved in the former method. After a single injection the plasma concentration of the test substance is rapidly falling, but none the less it is possible to obtain a correct clearance value provided that the correct methods for calculation are used. A practical problem is the urinary tract delay and although the correction for this may present some difficulties during the time period immediately after the injection of the test substance, the importance of the delay correction diminishes with time and the problem can be overcome by the use of long urine collection periods.

Another problem is the arterio-venous difference, which, however, can be completely avoided by the use of arterial blood.

For iothalamate in the dog the renal single injection method gives values that are equal to the continuous infusion values when arterial samples are used, while the arterio-venous difference should cause somewhat falling venous values. The total plasma clearance is higher than the continuous infusion clearance probably because the plasma curve has not reached its final slope after two hours, and the area is therefore underestimated, giving the too high clearance value.

For iothalamate in man the clearance values obtained by the renal single injection method are equal to those of the continuous infusion method when arterial samples are used and insignificantly higher when venous samples are used. The total plasma clearance is somewhat higher than the continuous infusion clearance; the reason might be the same as in the dog.

That the plasma curves of iothalamate have not reached their final slope after two hours is in accord with the fact that 35 - 40 per cent of the iothalamate is still retained in the body after two hours. A better agreement between the total plasma clearance and the continuous infusion clearance is therefore to be expected if the sampling period is prolonged.

Iodohippurate shows quite another behaviour in the dog as well as in man. After a single injection of this test substance the clearance values decrease from period to period, depending on a decreasing renal extraction, which in its turn is due partly to the presence of free iodine in the test substance and partly to the re-entry of the test substance from the kidney into the blood. Therefore, neither in the dog nor in man the renal single injection technique gives clearance values that are comparable to the continuous infusion clearance values. The two-hour total plasma clearance in the dog

is 22 per cent higher than the continuous infusion clearance but in man the two methods agree. The finding of a correct total plasma clearance in man is in all likelihood due to the fact that the iodohippurate is almost completely excreted after two hours and the plasma curve is thus complete. In the dog, on the other hand, more iodohippurate is retained after two hours, probably depending on a higher cellular uptake, and the curve is therefore not completely known, leading to an underestimation of the area and a corresponding overestimation of the clearance. In the case of iothalamate this can be compensated for by a prolongation of the sampling period, but this is not possible for iodohippurate since the disturbing influence of free iodine increases rapidly at low iodohippurate levels.

It may be concluded that correct calculation methods are now available for single injection clearance determinations, and for iothalamate, representing substances excreted by glomerular filtration, the renal single injection values are well comparable with the continuous infusion values. Correct total plasma clearance values can also be obtained if sufficiently long sampling periods are used. For iodohippurate, on the other hand, the renal single injection clearance values are not comparable to the continuous infusion values, due to the decreasing clearance after single injection of this type of test substance.

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The single injection technique for determination of renal clearance I.

Clearance of iothalamate and iodohippurate in dogs.

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The generally accepted method for performing renal clearance measurements is by way of the constant infusion technique with simultaneous urine collection in several periods. Standard substances are inulin for measurement of glomerular filtration rate (GFR) and para-aminohippuric acid (PAH) for measurement of renal plasma flow (RPF) (Smith, 1951, 1956).

During later years, however, inulin and PAH have been largely replaced by radioactive substances of different kinds, making the analysis of the samples simpler (Billion & Schlunbaum, 1955; Block & Burrows, 1958; Bergström, Bucht & Josephson, 1959; Denneberg, Ek & Hedenskog, 1961; Burbank, Tauxe, Maher & Hunt, 1961; Sigman, Elwood, Reagan, Morris & Cantanzaro, 1965; Stacy & Thorburn, 1966). The simultaneous determination of GFR and RPF by using inulin and PAH concomitantly has been replaced by the use of two differently labelled radioactive substances at the same time (Stokes & Ter-Pogossian, 1964; Cutler & Glatte, 1965; Farmer, Tauxe, Maher & Hunt, 1967; Elwood & Sigman, 1967).

It has been repeatedly suggested that the continuous intravenous infusion be replaced by a single intravenous injection of the clearance substance, but whether this method gives the same result as the continuous infusion method is widely discussed. Favourable reports are numerous (Alving & Miller, 1940; Foà & Foà, 1942; Landowne & Alving, 1946; Blaurox, Frohmüller, Campbell, Utz, Orvis & Owen, 1963; Meschan, Schmid, Watts & Witcofski, 1963; Greenlaw & Hudgins, 1964; Truninger, Donath & Kappeler, 1968; Ram, Holroyd & Chisholm, 1969; Cohen, Smith, Mindell & Vernier, 1969) but negative reports can also be found (Hilden, 1943; Hogeman, 1948; Hood, 1950; Josephson, 1947; Tacket & Houck, 1950; Smith, 1951; Dutz, 1952; Nüssgens & Fritz, 1956; Binnion & Cumming, 1967).

The differences of opinion seem largely to be due to variations in performance of the test and in calculation of the clearance values. Therefore it was decided to reinvestigate the problem of the single intravenous injection clearance and its relation to the continuous intravenous infusion clearance, using the methods recently devised by Nosslin (1965). Main emphasis was laid on the true renal clearance, as determined from the plasma curve and the urinary excretion but the plasma values were also used for estimation of the total clearance. Among substances suggested for measurement of GFR sodium iothalamate was selected (Sigman et al, 1965; Griep & Nelp, 1969). As a substitute for PAH sodium ortho-iodo-hippurate was chosen, since it is not protein-bound or taken up by the liver to the same degree as iodopyracet (Block & Burrows, 1960; Whitley, Witcofski & Meschan, 1961). Both substances are commercially available, labelled with either ^{125}I or ^{131}I .

MATERIAL AND METHODS

Animals. Eleven female mongrel dogs weighing 12-23 kg were used. They were kept on the ordinary diet of the laboratory. After a short period of training they were able to stand loosely supported in a special frame during the clearance measurements. During at least twelve hours before each experiment they were given no food but allowed water ad libitum.

Test substances. Sodium iothalamate and sodium ortho-iodohippurate, both labelled with either ^{125}I or ^{131}I , were purchased from the Radiochemical Centre, Amersham, England and AB Atomenergi, Studsvik, Sweden, respectively. After appropriate dilution they were kept in the dark in the refrigerator. Substances labelled with ^{131}I were never kept for more than two weeks. For every experiment 12 - 20 μCi of each substance was used, corresponding to an amount of 2 - 4 mg iothalamate and 0.4 - 2 mg iodohippurate. The single injection dose was made up by diluting one or both of the test substances with physiologic saline in a sterile vial to a volume of 10 ml, of which 8 ml constituted the injection dose. Of the remaining solution 1 ml was further diluted to a volume of 100 ml, and 1 ml of this solution was used for counting and calculation of the total dose given to the animal. The substance intended for continuous infusion was diluted with physiologic saline to a volume of 8 ml. One or 2 ml were given as a priming dose and the rest was added to 600 ml of physiologic saline and used for infusion.

Experimental procedure. In order to obtain a good diuresis the dog was given 80 ml per kg bodyweight of warm tapwater by stomach tube. One hour later the dog was placed in the frame, an intravenous cannula was inserted into a foreleg vein, and an intravenous plastic catheter was put into a hindleg vein. A bladder catheter (usually Charrière 14) was applied with the help of a speculum and fixed with adhesive tape. Control samples of urine and blood were taken and after injection of the priming dose the continuous infusion was started, being given in the foreleg cannula by an infusion pump at a rate of 2.8 ml/min. When both test substances were given by single injection, saline only was given as continuous infusion.

One hour after the beginning of the continuous infusion the urinary bladder was emptied, rinsed with saline and air, and the single intravenous in-

jection was given. At 3, 6, 10, 15, 30, 50, 70, 90 and 120 minutes blood samples of 5 ml were drawn into heparinized syringes from the hindleg catheter, care being taken to discard the content of the catheter each time. The total blood loss for the animal during one experiment was about 60 ml, corresponding to a mean plasma 'clearance' of 0.02 ml/min/kg. The blood samples were centrifuged at 3800 rpm during five minutes and the plasma was separated. Four urine collection periods of 20 minutes each were run, the bladder being flushed with 50 ml of saline and then repeatedly with air at the end of each period. Urine and irrigating fluid were combined after the volumes had been measured. At the end of the experiment 1 ml aliquots of all plasma and urine samples were taken by constriction pipettes into special plastic tubes for counting. Hematocrit was determined at the beginning and at the end of the experiment.

Series. Three separate series were run. In Series I ^{125}I -iothalamate and ^{131}I -iodohippurate were both given as a single injection. In Series II ^{131}I -iothalamate was given as a single injection and ^{125}I -iothalamate as a continuous infusion. In Series III ^{125}I -iodohippurate was given as a single injection and ^{131}I -iodohippurate as a continuous infusion. After discarding experiments where the urine production in any period was less than 1 ml/min, or where technical mishaps had occurred, there remained 31 experiments in Series I, 15 in Series II and 14 in Series III.

Measurement of radioactivity. The radioactivity was measured in 1 ml samples of plasma and urine in a Packard automatic gamma spectrometer model 5212 with window settings 15 - 60 keV (window A) and 200 - 460 keV (window B). After correction for background activity the count in window B denoted only the activity of ^{131}I in the sample. The true activity of ^{125}I was calculated by subtracting from the corrected count in window A, the count of ^{131}I in window B multiplied by the quotient found between the two channels on counting of an ^{131}I standard. In single injection experiments the background activity and the maximum and minimum uncorrected sample activities were about 80, 6000 and 850 cpm, respectively, in window A, and about 120, 6000 and 300 cpm in window B. A fixed counting time of 10 minutes was used, resulting in a counting error, as calculated according to formulas given by Ambrosen (1967), of less than 2 % for ^{125}I and less than 3.6 % for ^{131}I . In continuous infusion experiments the uncorrected sample activities were around 1200 cpm in window A and around 350 cpm in window B, giving counting errors of 1.3 % for ^{125}I and 3 % for ^{131}I .

Calculations. The continuous infusion clearance was calculated according to the standard formula:

$$C = \frac{U \times V}{P} \quad (1)$$

where U = cpm per ml urine, P = cpm per ml plasma, and V = urine flow in ml per minute.

The single injection clearance was calculated according to the formula:

$$C = \frac{E}{A} \quad (2)$$

where E = total amount excreted in urine (cpm) during a certain time, and A = area under the plasma curve (cpm/ml x time) during the corresponding time.

Formulas (1) and (2) can both be derived from the following simple identity:

Amount cleared from plasma by the kidneys = Amount excreted in urine

which is valid independently of the mode of administration of the test substance. Consequently, for a time period t with a mean concentration $\bar{P}$ in plasma:

$$C \text{ (ml/min)} \times \bar{P} \text{ (cpm/ml)} \times t \text{ (min)} = U \text{ (cpm/ml)} \times V \text{ (ml/min)} \times t \text{ (min)} \quad (3)$$

For continuous infusion, where the plasma concentration is constant, $\bar{P}$ is identical with P and after division by t on both sides we get formula (1). For single injection the mean concentration can be written as the area under the plasma curve divided by time:

$$\bar{P} = \frac{\int_{t_1}^{t_2} P \, dt}{t} = \frac{A}{t}$$

where $t = t_2 - t_1$. Substitution in formula (3) gives formula (2):

$$C = \frac{U \times V \times t}{A} = \frac{E}{A}$$

The formula for total clearance (total plasma clearance) can be derived from formula (2) by letting E denote the total excretion by all routes. After

infinite time the total dose must have been excreted, giving the expression:

$$C = \frac{D}{A} \quad (4)$$

where D is dose (cpm) and A is total area under the plasma curve (cpm/ml $\times$ time).

A similar derivation of the clearance formulas was given by Nosslin (1965).

In the calculation of the single injection clearance the delay that occurs in the urinary tract, which functions as a dead space, must be taken into account. Therefore, in formula (2), the excreted amount (E) must be divided by the area under the plasma curve (A) for a somewhat earlier time period. In the present experiments a delay time correction of two minutes was applied, which means that the time periods 0 - 20, 20 - 40, 40 - 60, and 60 - 80 minutes were used for collection of urine and the corresponding time periods for the area under the plasma curve were 0 - 18, 18 - 38, 38 - 58, and 58 - 78 minutes (Fig 1). A clearance value was calculated not only for each twenty-minute period, but also for a total period 0 - 80 (0 - 78) minutes. This mean value is consequently not identical with the arithmetic mean of the four clearance periods.

The area under the plasma curve was obtained by planimetry in duplicate after drawing of the curve in linear scale by fitting a line as smooth as possible to the known points. For the first three minutes the part of the area that was below the three-minute value was determined by planimetry, while the part of the area that was above the three-minute value was calculated as half of the area under a straight line connecting a theoretical initial value with the three-minute value. The theoretical initial value was calculated as the quotient between the dose given and the estimated plasma or blood volume. Plasma volume and blood volume were taken as 50 and 80 ml/kg bodyweight, respectively (Seifert, Jesch, Klövekorn & Messmer, 1972). In seven experiments the true area under the initial plasma curve was determined by planimetry of curves obtained by frequent sampling during the first minutes. The results did not differ significantly from those obtained by calculation according to the above-mentioned method.

To obtain the remaining area, to infinity, the plasma value at 78 minutes was divided by the assumed final slope of the plasma curve as calculated with the least-square technique from the plasma values at 70, 90 and 120 minutes. This area was added to the area obtained by planimetry (0 - 78 minutes), giving the total area under the plasma curve.

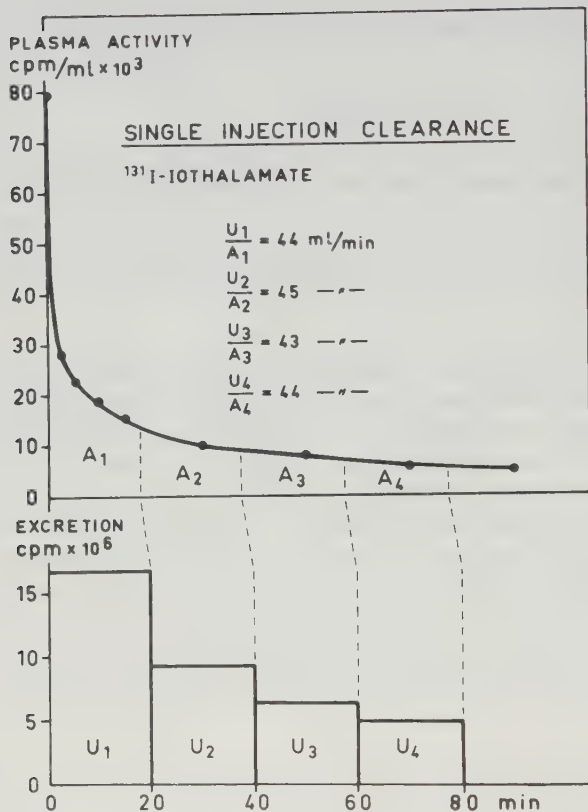


Fig 1 Results of plasma and urine measurements in an experiment with single injection of ^{131}I -iothalamate. The calculation of the clearance values from the urinary excretion and the corresponding part of the area under the plasma curve (after correction for urinary tract delay) is also shown.

Statistical methods. Statistical comparisons were based on Student's t-test for paired observations and on analysis of variance for groups of observations. Regression within groups was studied with analysis of covariance, otherwise standard statistical procedures were used (Snedecor, 1955). Differences found were considered probably significant if $P < 0.05$ and significant if $P < 0.01$.

RESULTS

The animals tolerated the procedure well and they were usually at ease during the experiment. During continuous infusion the plasma levels of iothalamate as well as those of iodohippurate did not show any significant trend in their variation and the levels in no case deviated more than ten per cent from the mean level during the experiment.

The results of the clearance measurements are presented in Table I and in Figs 2, 3, and 4.

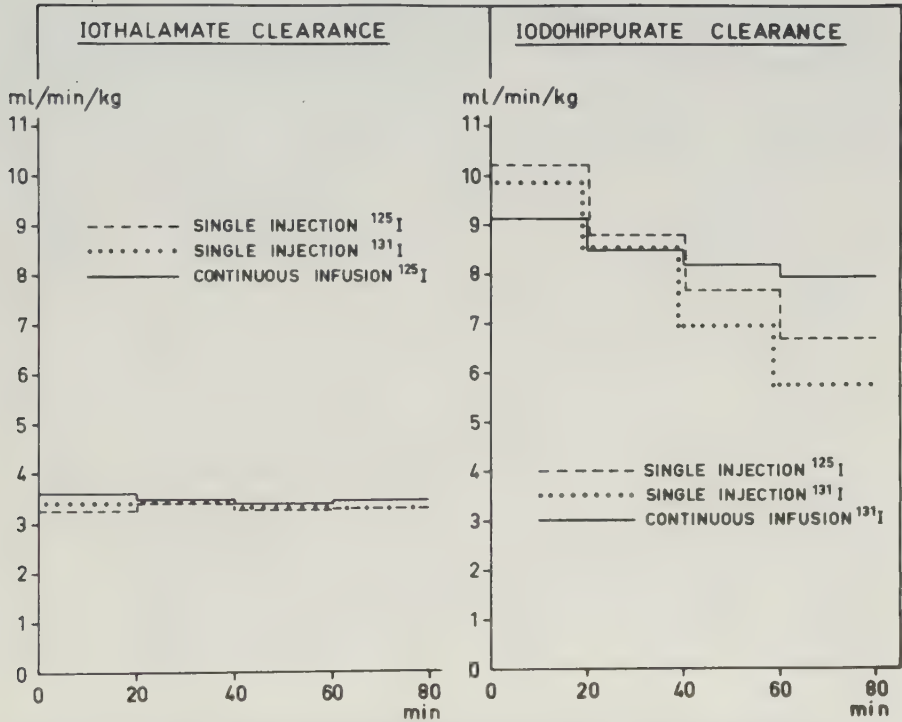


Fig 2 Results of single injection and continuous infusion clearance determinations with iothalamate and iodohippurate.

Iothalamate. The continuous infusion clearance was on the average 3.5 ml/min/kg. The error of the method was 5.4 % for a single period, corresponding to 2.7 % for a mean of four periods.

Table I. Results of clearance measurements

Method	Iso- tope	Number of experi- ments	Clearance periods				Mean clearance		Total clearance m	Regression		Error of method ^b
			I	II	III	IV	m	SD		Coefficient ^a	Change/period	
<u>Iothalamate</u>												
Single injection. Series I	125 _I	31	3.24	3.38	3.26	3.27	3.28 ± 0.49	3.70 ± 0.52	- 0.0001 ⁻	- 0.1	7.6	
Single injection. Series II	131 _I	15	3.39	3.42	3.28	3.27	3.38 ± 0.32	3.83 ± 0.41	- 0.0024 ⁻	- 1.4	6.5	
Continuous infusion. Series II	125 _I	15	3.60	3.45	3.39	3.46	3.47 ± 0.32		- 0.0025 ⁻	- 1.3	5.4	
<u>Iodohippurate</u>												
Single injection. Series I	131 _I	31	9.86	8.54	6.95	5.74	8.88 ± 1.36	8.42 ± 1.05	- 0.0703 ^{**}	- 15.8	11.5	
Single injection. Series III	125 _I	14	10.20	8.81	7.69	6.69	9.40 ± 1.12	10.06 ± 1.20	- 0.0583 ^{**}	- 12.4	7.5	
Continuous infusion. Series III	131 _I	14	9.12	8.51	8.21	7.95	8.44 ± 1.38		- 0.0190 ^{**}	- 4.5	6.5	

^a Significance denoted by - for P > 0.05, * for P < 0.05 and ** for P < 0.01^b Coefficient of variation for a single period clearance value.

The renal single injection clearance was 3.3 ml/min/kg, irrespective of type of label used, and the period values showed a slight and insignificant decrease with time. The error for a single period was about 7 %. In Series II a direct comparison between simultaneous determinations showed that the mean single injection value was very close to the continuous infusion value. The agreement in the individual case was also good (Fig 3) and the correlation coefficients were 0.98 for clearance expressed as ml/min and 0.94 for clearance expressed as ml/min/kg.

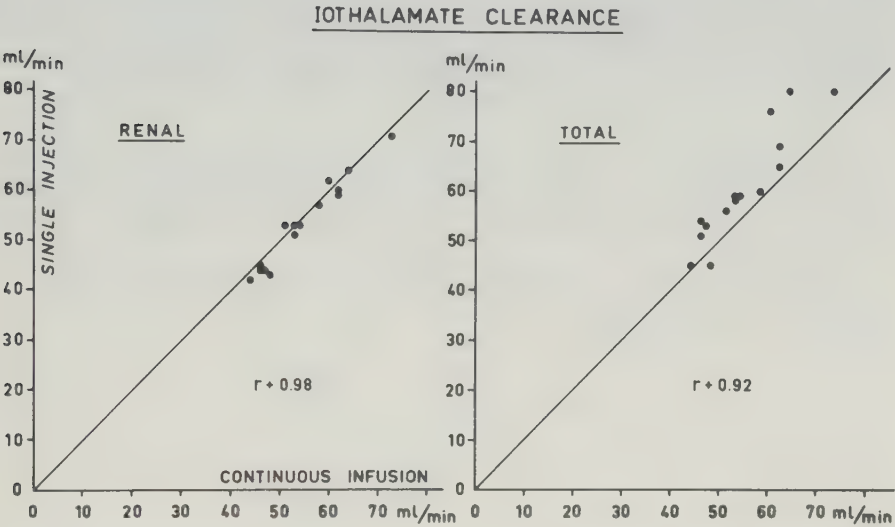


Fig 3 Correlation between simultaneous determinations of the iothalamate clearance with the single injection and the continuous infusion method in 15 dogs (Series II). Line of identity inserted.

The total clearance was 10 - 13 % higher than the simultaneous renal clearance and a comparison with the continuous infusion clearance in Series II (Fig 3) gave correlation coefficients of 0.92 and 0.75 for clearance expressed as ml/min and ml/min/kg, respectively.

Iodohippurate. The results for this test substance were rather different from those of iothalamate. During continuous infusion the clearance was gradually decreasing with a mean change of 4.5 % per period. After single injection a much greater fall was found, amounting to 12 and 16 % per period in the two series studied. The greatest fall was found when ^{131}I -labelled iodohippurate was used. The individual period values were higher than the continuous infusion values during the initial period, identical during the second period and progressively lower in later periods. The mean values were 8.5 ml/min/kg for continuous infusion and 8.9 and 9.4 ml/min/kg after single injection. The error for a single period determination was 6.5 % for continuous infusion and 7.5 and 11.5 % for single injection. In Fig 4 it can be seen that the single injection values were systematically higher than the simultaneous continuous infusion values and that the correlation was not as good as for iothalamate. The correlation coefficients were 0.71 and 0.91 for clearance expressed as ml/min and ml/min/kg, respectively.

The total clearance was somewhat lower than the simultaneous renal single injection clearance in Series I, but 19 % higher than the concomitant continuous infusion clearance in Series III (Fig 4). The coefficients for the correlation between the total clearance and the continuous infusion clearance were 0.62 and 0.86 for clearance expressed as ml/min and ml/min/kg, respectively.

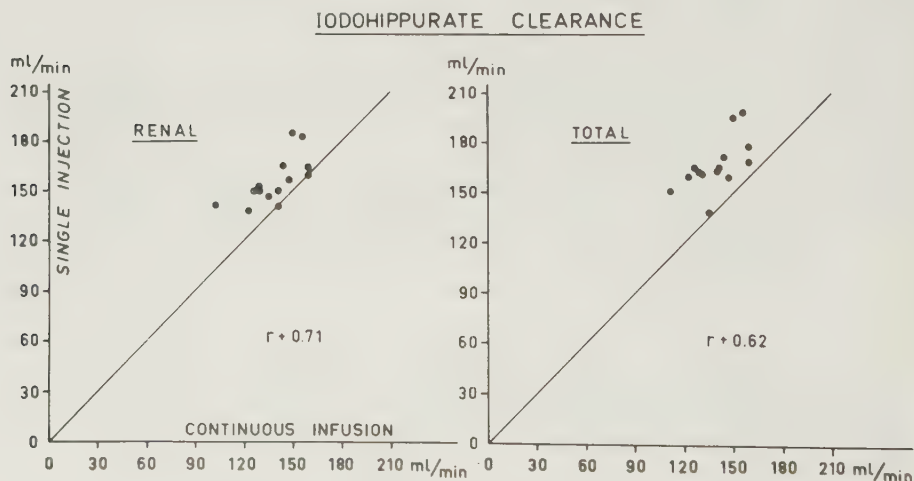


Fig 4 Correlation between simultaneous determinations of the iodohippurate clearance with the single injection and the continuous infusion method in 14 dogs (Series III). Line of identity inserted.

DISCUSSION

The total clearance of iothalamate as well as of iodohippurate was considerably higher than the concomitant continuous infusion clearance. This can hardly be due to an extrarenal clearance since this is known to be insignificant for both test substances in the presence of normal kidneys (Griep & Nelp, 1969; Magnusson, 1962). A possible explanation is that the area under the plasma curve, being the only variable in the formula for calculation of the total clearance, has been calculated too small. Since the main part of this area was directly measured by planimetry the error must be attributed to the estimation of the remaining part of the area, the magnitude of which depends on the slope of the plasma curve. It must be concluded, therefore, that the plasma curve had not reached its final slope during the time interval 70 - 120 minutes.

The renal clearance values for iothalamate were very nearly the same whether determined by the single injection technique or by the continuous infusion technique, and the errors of the two methods were about equal.

For iodohippurate the results were quite different in that a remarkable fall in the renal clearance values from period to period especially after single injection but also during continuous infusion was found. A similar decrease in the continuous infusion clearance values was registered by Deyrup (1947) in dogs that were kept standing but not in dogs that were lying down during the test. A gradual fall in the clearance values of dogs during continuous infusion noted by Smith (1951) was considered due to fatigue and decreasing hydration. One or both of the above-mentioned explanations may be applicable in the present investigation, where the iothalamate clearance also showed some decrease, although not significant. The difference in decrease between iothalamate and iodohippurate might be due to the presence of free iodine in the latter. The decrease in the clearance values after single injection of iodohippurate was much more impressive and amounted to eight and eleven per cent per period in the two series run even after correction for the decrease found during continuous infusion.

It seems then that the single injection method works well when used for renal clearance determinations with iothalamate. The corresponding iodohippurate results, however, indicate either that the method does not work with this substance or that the substance has properties that lead to a decreasing clearance. Some known properties of iodohippurate make the latter explanation more probable. So for instance there is some free iodine in all radioactive preparations of iodohippurate and since the

clearance of iodine is very low compared to that of iodohippurate the relative concentration of free iodine will increase with time and the clearance values will consequently drop. Opinions differ about the extent of influence on the clearance values (Burbank et al.; 1961, Scholz & Oeff, 1963; Meschan et al., 1963; Bianchi, Hegesippe, Meozzi, Rosa & Sosi, 1965). Because of the higher radiation energy of ^{131}I there is more autoradiolysis in compounds with this isotope than in those with ^{125}I , and the difference between the results in the two iodohippurate single injection series may depend on different content of free iodine.

Another factor that might explain the fall in the clearance values is protein-binding of the test substance. It is well known that the clearance of iodothyronine falls from period to period when given as a single injection and this has been attributed to protein-binding (Hogeman, 1948; Block & Burrows, 1958, 1960). Concerning the protein-binding of iodohippurate widely divergent results have been published. Smith & Smith (1938) found 70 % to be bound in man and 78 % in the dog, while Magnusson (1962) found practically no binding in man. Kimbel (1963) stated that 40 to 60 % was protein-bound, dependent upon the concentration.

An uptake of iodohippurate into the red blood cells is known to occur but again there are differences of opinion as to the extent of the uptake (Smith, Finkelstein, Aliminosa, Crawford & Graber, 1945; Burbank et al., 1961; Magnusson, 1962), and the degree of influence on the clearance values is unknown.

A resorption from the urinary tract and recirculation of the iodohippurate might cause diminishing clearance values. Whether such a resorption exists for iodohippurate is not known but Heidenreich, May & Kook (1962) found a considerable resorption of PAH from the dog ureter.

Very little is known about the renal extraction of clearance substances during falling plasma levels, but Block & Burrows (1960) found diminishing extraction of iodothyronine with decreasing plasma levels, and Sieberth & Mertz (1965) found an extraction of only 56 % in dogs after a single injection of iodohippurate. A decreasing extraction rate would explain the finding of a decreasing clearance.

The use of venous blood in the present investigation may have influenced the clearance values. Brun, Hilden & Raaschou (1949) found that with rapidly falling iodothyronine and inulin plasma levels the concentration of the test substance was higher in the venous blood than in the arterial blood and the average difference was 29 % for iodothyronine and 7 % for inulin. The use of venous blood under these circumstances lead to erroneously low clearance values.

The importance of an adequate delay time correction was also pointed out by Brun et al.(1949) and they recommended a considerably greater correction than that usually applied. The time correction used in the present investigation was two minutes and may not have been quite adequate.

Although several factors may influence the renal clearance values after single injection of the test substance, we believe that the method is correct in principle and that the clearance of iodohippurate actually decreases due to one or more of its above-mentioned properties. Conn, Sabo, Landes & Ho (1964) and Landes, Sabo, Conn, Milam & Green (1966) who also found a decreasing iodohippurate clearance offered a kinetic explanation, but further investigations seem necessary to solve the problem.

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The single injection technique for determination of renal clearance II.

Experimental studies on methodological problems.

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In a previous investigation a comparison was made between the single injection technique and the standard, continuous infusion technique for performing renal clearance determinations, using radioiodinated sodium iothalamate and sodium ortho-iodohippurate as test substances (Pihl & Nosslin, 1973). A pronounced fall of the clearance values from period to period was found for iodohippurate, and various factors that might account for this phenomenon were discussed, such as the occurrence of free iodine, protein-binding, red blood cell uptake, and resorption from the urinary tract. Also the use of venous instead of arterial blood and the application of an incorrect delay-time were mentioned as possible explanations.

The aim of the present investigation was to elucidate the influence of these factors on the single injection clearance values not only of iodohippurate but also, for the sake of comparison, of iothalamate. In some respects ^{51}Cr -EDTA, which has been more and more used for clearance measurements (Stacy & Thorburn, 1966; Garnett, Parsons & Veall, 1967; Favre & Wing, 1968; Bröchner-Mortensen, Giese & Rossing, 1969; Aurell & Ditzel, 1970) was also investigated.

MATERIAL AND METHODS

Animals. Sixteen female, mongrel dogs weighing 13 - 25 kgs were used. They were kept on the ordinary diet of the laboratory. After a short period of training they were able to stand loosely supported in a special frame during the clearance measurements. During at least twelve hours before each experiment they were given no food but allowed water ad libitum.

Patients. In connection with clearance determinations on twenty-nine patients from the Department of Urology the red blood cell uptake in vivo, the arterio-venous difference and the influence of carrier was also investigated. The patients were of both sexes and varied in age between 21 and 79 years.

Test substances. Sodium iothalamate and sodium ortho-iodohippurate labelled with ^{125}I or ^{131}I and Cr-EDTA labelled with ^{51}Cr were purchased from the Radiochemical Centre, Amersham, England. After appropriate dilution they were kept in the dark in the refrigerator. Substances labelled with ^{131}I were never kept for more than two weeks. Each

batch of the iodine-labelled substances was checked for free iodine by chromatography. For iodohippurate this was performed as ascending paper chromatography on Whatman No 1 filter paper with butanol - acetic acid - water (4:1:1) as solvent. The activity was localized with autoradiography. For iothalamate thin layer chromatography on Merck F₂₅₄ silica gel plates with butanol - acetic acid - water (75:12:30) as solvent was used. The plates were scanned in a Packard radiochromatogram scanner model 7201.

For each clearance experiment 20 - 100 μCi (in the case of ^{51}Cr -EDTA up to 300 μCi) of the test substances were used, corresponding to 0.2 - 20 mg of iothalamate, 0.1 - 10 mg of iodohippurate and less than 1 mg of Cr-EDTA. The single injection dose, the priming dose and the dose standard were made up as earlier described (Pihl & Nosslin, 1973).

Experimental procedure for clearance measurement. The animal clearance measurements were carried out under intravenous sodium pentobarbital anesthesia with endotracheal intubation when arterial catheters were used for blood sampling. The induction dose was 25 mg per kg bodyweight and small supplementary doses were given as needed. The dogs were tied in a supine position on an animal board and a bladder catheter (usually Charrière 14) was applied with the help of a speculum and fixed with adhesive tape. Control samples of urine and blood were taken and 400 - 500 ml of physiologic saline with 5 % mannitol was administered intravenously. After injection of the priming dose the continuous infusion, also containing 5 % mannitol, was started, being given by an infusion pump at a rate of 2.8 ml/min. When both substances were given by single injection, saline with 5 % mannitol was given as a continuous infusion. With the Seldinger technique an ordinary thick-walled red Ödman-Ledin catheter was placed in the femoral artery. When venous blood was also used a similar catheter was introduced into a hindleg vein. One hour or more after the beginning of the continuous infusion, the urinary bladder was emptied, rinsed with saline and air, and the single injection was given. At 1, 3, 6, 10, 15, 30, 50, 70, 90, 110 and 130 minutes blood samples of 5 ml were drawn into heparinized syringes, care being taken to discard the content of the catheter each time. If venous as well as arterial blood was obtained the samples were drawn simultaneously. The total blood loss for the animal during one experiment was about 130 ml, corresponding to a mean plasma 'clearance' of 0.04 ml/min/kg. In most cases whole blood as well as plasma was obtained. Centrifugation at 3800 rpm for five minutes was performed immediately and the plasma was pipetted off without delay.

Four to six urine collection periods of 20 minutes each were run, the bladder being flushed with 50 ml of saline and then repeatedly with air

at the end of each period. Urine and irrigating fluid were combined after the volumes had been measured. At the end of the experiment 1 ml aliquots of all blood, plasma and urine samples were taken by constriction pipettes into special plastic tubes for counting. Hematocrit was determined at the beginning and at the end of the experiment.

The procedure for unanesthetized animals was very similar and has been described in detail earlier (Pihl & Nosslin, 1973). For clearance determinations in patients the procedure was in principle as for the animals and only differences are described. The patients were allowed a light breakfast and were given about 500 ml of water orally in the morning. They were kept in bed. Most patients had an indwelling Foley catheter, which was usually replaced by a sixhole catheter. The single injection, the priming dose and the continuous infusion were given through an indwelling intravenous cannula in an arm vein. The continuous infusion fluid was in most cases 5 % glucose and the rate of administration was 5.4 ml/min. Blood samples were obtained through plastic catheters placed in the femoral artery and vein by the Seldinger technique under local anesthesia. Usually six clearance periods were run, the bladder being flushed with 2 x 30 ml of saline and air between each period.

Influence of free iodine. The influence of free iodine on the clearance values was studied in two series of dogs. In the first series ten animals underwent clearance determinations four to seven times each with iodohippurate given by single injection. In the second series nine animals had clearance measurements with iodohippurate given by continuous infusion three to seven times each. The free iodine content of the iodohippurate was known for every test and varied between 0.7 % and 6.0 %. For the two series within-group correlation between free iodine content and the mean clearance values as well as the quotient between clearance values for the first and last periods was calculated using analysis of covariance (Snedecor, 1955).

Influence of carrier. The influence of carrier was studied in three series of paired experiments in dogs, where the same dog underwent clearance determination with the same test substances with as well as without carrier. Series I consisted of six dogs under anesthesia given ^{125}I -iothalamate by single injection and ^{131}I -iothalamate in a continuous infusion. As carrier was used Conray⁽³⁾, containing 0.5 g sodium iothalamate per ml. Two ml were given as a priming dose and 8 ml was added to the continuous infusion fluid (1000 ml) giving calculated plasma levels of 20 - 30 mg %. Whole blood as well as plasma was obtained. In Series II nine dogs, two

of which were under anesthesia, were given ^{125}I -iodohippurate by single injection and ^{131}I -iodohippurate in continuous infusion. As carrier was used commercial ortho-iodohippuric acid (Hopkin & Williams, Ltd., England) prepared for intravenous use by the hospital pharmacy. The priming dose was 0.1 - 0.2 g and 1 - 1.5 g was added to the continuous infusion fluid (1000 ml) giving calculated plasma levels of 2 - 3 mg %. Whole blood and plasma was obtained. The five dogs in Series III were anesthetized and all underwent clearance measurements with ^{125}I -iodohippurate given as a continuous infusion, but only three of them had clearance determinations with ^{125}I -iodohippurate as a single injection. The carrier was as in Series II. Only whole blood was obtained.

Clearance measurements with ^{125}I -iodohippurate given as a single injection and carrier iodohippurate given in a continuous infusion were also performed on three male patients, aged 59, 61 and 64 years. The kidneys were normal in two of the patients and in the third there was one small renal cyst on each side. On three other male patients, aged 57, 64 and 74 years, single injection clearance determinations with iodohippurate without carrier was performed. One of these patients had normal kidneys and the other two each had one small cyst in one kidney.

Uptake into red blood cells. Studies in vitro were performed on dog blood as well as on normal human blood. Heparin was used as anticoagulant. Two types of experiments were run. In type I experiments the shift from plasma to red blood cells was studied, and in type II experiments the shift from red cells to plasma was investigated. In type I about 80 ml of blood was transferred immediately after drawing to a round glassflask, which was kept under constant rotation in a waterbath at 37°C . At zero-time 3 - 10 μCi of the test substance (or substances) was added by a syringe and thereafter blood samples were taken through a plastic catheter at 1, 2, 4, 7, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150 and 180 minutes. The samples were 4 ml except at 1, 2, 150 and 180 minutes when 6 ml were taken. The latter samples were divided before centrifugation so that whole blood as well as plasma was obtained. Centrifugation at 3800 rpm for five minutes was done immediately and the plasma was separated at once. For counting purposes 1 ml aliquots were taken by constriction pipettes. Hematocrit was determined on the two first and the two last samples. Of type I experiments four with ^{125}I -iothalamate and ^{51}Cr -EDTA together and six with ^{125}I -iodohippurate only were run on dog blood. On human blood six experiments with ^{125}I -iothalamate and ^{51}Cr -EDTA and four with ^{125}I -iodohippurate only were performed.

In type II experiments 160 ml of blood was used. The blood was divided into two equal portions, one of which was transferred to a round glassflask,

which was kept under constant rotation in a waterbath at 37°C. About 25 μCi of the test substance was added to the flask. After centrifugation of the second portion the plasma was separated and kept in the waterbath at 37°C. Three hours later control samples were taken from the whole blood, which was then centrifuged and the plasma separated and discarded. The plasma from the second portion and the blood cells from the first portion were then instantly mixed in a glassflask kept under constant rotation in the waterbath at 37°C. Blood samples were taken and treated as in type I experiments. Type II experiments were performed with ^{125}I -iodohippurate only and four experiments on dog blood and four on human blood were run.

Four dogs were used for investigation of the influence of delay between the drawing and the centrifugation of a blood sample. After a single injection of 50 μCi of ^{125}I -iodohippurate 30 ml of blood was drawn, in dog A after 1 minute, in dog B after 3 minutes, in dog C after 10 minutes, and in dog D after 30 minutes. The blood was equally divided between six tubes, one of which was kept as whole blood, while the others were centrifuged after 0, 5, 10, 20 and 40 minutes, respectively, and the plasma immediately separated. Aliquots of 1 ml were taken by constriction pipettes for counting.

Studies of the red cell uptake in vivo were performed on dogs and in patients undergoing clearance determinations with the single injection technique as well as with the continuous infusion technique. The blood samples were divided before centrifugation so that whole blood as well as plasma was obtained. The centrifugation was performed immediately and the plasma was pipetted off within six to seven minutes from the drawing of the sample. The number of experiments are given in Fig 3.

The red cell uptake of iodohippurate was also calculated for arterial as well as for venous blood on the dogs and the patients where simultaneous arterial and venous blood samples were taken during clearance determinations.

Resorption from the urinary bladder. In two dogs 50 μCi of ^{125}I -iothalamate and 50 μCi of ^{131}I -iodohippurate were mixed with 50 ml urine and instilled into the bladder. Blood samples were obtained at 5, 10, 15, 20, 30 and 50 minutes.

Arterio-venous difference. The difference between arterial and venous blood concentration of the test substances was investigated in dogs and

patients undergoing clearance measurements with the combined single injection and continuous infusion techniques. Simultaneous blood samples were obtained from catheters in the femoral artery and a hindleg vein in dogs and from the femoral artery and vein in patients. Plasma as well as whole blood was measured. The studies were performed on six dogs and nine patients with ^{125}I -iothalamate, three dogs with ^{51}Cr -EDTA (single injection only), and six dogs and eight patients with ^{125}I -iodohippurate.

Delay time. The urinary tract delay was investigated by clearance determinations with frequent and short urinary collection periods in six dogs. They were prepared as for ordinary clearance measurement except that a two-way urinary catheter was applied, through which the bladder was continuously irrigated with saline given at a constant rate. About 50 μCi of ^{125}I -iothalamate was given as a single injection. Urine and irrigating fluid was allowed to drain freely through the catheter and the collection periods were three minutes during the first 15 minutes, five minutes between 15 and 40 minutes, and ten minutes between 40 and 120 minutes. Blood samples were drawn and treated as in ordinary clearance experiments. Three of the experiments had to be discarded because of too much variation in clearance values between periods. In the remaining three experiments the delay time was estimated according to a principle illustrated in Fig 1. For each experiment two cumulative curves were constructed

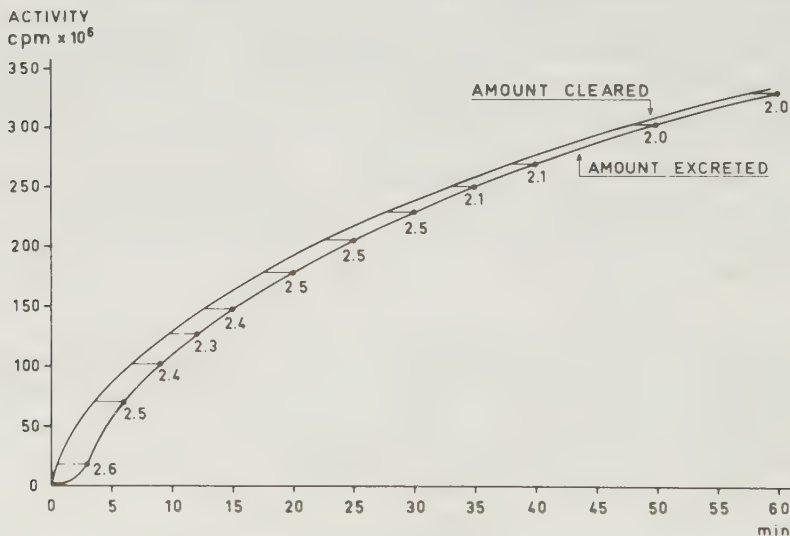


Fig 1 Illustration of the principle for estimation of delay time from the dislocation in time between the clearance curve and the excretion curve.

ted, one giving the amount of the test substance cleared from plasma and the other the amount recovered in the urine. Mathematically, the two curves correspond to $C \times A$ and E , respectively, in the formula for calculation of the clearance. In the absence of urinary tract delay the two curves should coincide since all of the test substance cleared from plasma should be immediately recovered in the bladder. The delay actually present is represented by the horizontal dislocation of the excretion curve in relation to the clearance curve, and can be read off from the diagram. The clearance value used in the calculation was the value of the total period 0 - 120 min. without any delay correction, which at this period-length is insignificant.

Measurement of radioactivity. The radioactivity was measured in 1 ml samples of plasma or blood and urine in a Packard automatic gamma spectrometer model 5212 using standard dual channel technique, as earlier described (Pihl & Nosslin, 1973). For measurement of ^{51}Cr the window setting was the same as for ^{131}I . A fixed counting time of 10 minutes was used.

For in vivo experiments, the counting error as calculated according to formulas given by Ambrosen (1967) was in general less than 2 % for ^{125}I , less than 3.6 % for ^{131}I and less than 4.5 % for ^{51}Cr . In the in vitro experiments the sample activity was considerably higher and the counting error consequently much smaller.

Calculations. The continuous infusion clearance was calculated according to the standard formula:

$$C = \frac{U \times V}{P}$$

where U = cpm per ml urine, P = cpm per ml plasma or blood, and V = urine flow in ml per minute.

The single injection clearance was calculated according to the formula:

$$C = \frac{E}{A}$$

where E = total amount excreted in urine during a certain time, and A = area under the plasma curve during the corresponding time period (Pihl & Nosslin, 1973). In the dog experiments a delay time correction of two minutes was used except in the experiments on delay time. For patients the delay time was calculated according to a formula given by Nosslin (1965):

$$T = 3.6 + 2.6/V$$

where T = delay time in minutes and V = urine flow in ml per minute. Usually the delay amounted to 4 minutes. A clearance value was calculated not only for each 20-minute period but also for the total period 0 - 80 or 0 - 120 minutes. This mean value is consequently not identical with the arithmetic mean of the four or six clearance periods. In the experiments concerning arterio-venous difference clearance values were also calculated for increasing period lengths from 20 minutes to 120 minutes.

The uptake into red blood cells in vitro was calculated in the following way in type I experiments: From a mean hematocrit (Hct) and a mean initial concentration in whole blood (B_o), calculated from the two first and the two last samples, a theoretical initial plasma concentration (P_o) was obtained: $P_o = B_o \times \frac{100}{100-Hct}$. All measured plasma concentrations were then expressed as a percentage of this value, and the concentration in the red blood cells (R) was obtained by subtracting this value from 100 since the activity not present in the plasma must be in the cells. To eliminate differences due to different hematocrit in the experiments, the red blood cell value was corrected to a standard hematocrit of 45 % for the dog and 43 % for man by multiplication with the factor $\frac{45}{Hct}$ and $\frac{43}{Hct}$, respectively.

In type II experiments mean hematocrit and mean initial whole blood concentration was calculated as above. A theoretical initial value for red blood cells (had all activity been in the cells), was then obtained:

$R_o = B_o \times \frac{100}{Hct}$ and all measured plasma values were recalculated to red blood cell values, which were then expressed as a percentage of the theoretical initial value and corrected with the factor $\frac{45}{Hct}$ and $\frac{43}{Hct}$ for the dog and for man, respectively.

For the in vivo experiments mean hematocrit was calculated from the values obtained at the beginning and at the end of the experiment, and the theoretical initial plasma and whole blood values were obtained by dividing the dose given by the estimated plasma and blood volume, respectively. The plasma and blood volumes were taken as 50 and 80 ml/kg for the dog and 43 and 75 ml/kg for man. The plasma and red blood cell activity in the samples were then expressed as a percentage of the whole blood activity at the time they were taken. Hematocrit correction was done as described for in vitro experiments. To determine the rate of exchange

the percentage of activity in plasma and red blood cells was expressed as concentration, and since the water content of plasma and red blood cells is 93 % and 70 % respectively (Ponder, 1949), the plasma values were divided by $0.93 \times \frac{100 - \text{Hct}}{100}$ and the red blood cell values by $0.70 \times \frac{\text{Hct}}{100}$. The difference between the concentrations in plasma and in red blood cells was then plotted against time on a semilogarithmic diagram. The initial decrease rate of the curve was determined from the slope of the tangent at zero time and expressed as per cent decrease per minute.

Statistical methods. Statistical comparisons were based on Student's t-test for paired observations and on analysis of variance for groups of observations. Regression within groups was studied with analysis of covariance, otherwise standard statistical procedures were used (Snedecor, 1955). Differences found were considered probably significant if $P < 0.05$ and significant if $P < 0.01$.

RESULTS

Influence of free iodine. For iothalamate the chromatograms never showed free iodine in excess of 1.0 %. For iodohippurate the results varied between 0.2 % and 2.0 % for ^{125}I and 0.4 % and 6.4 % for ^{131}I . Mean values were 1.0 % and 2.6 %, respectively. In the single injection series no correlation could be demonstrated between the mean clearance values or the quotients between the first and last period values and the content of free iodine in the iodohippurate used. In the continuous infusion series, however, there was a significant correlation with the mean clearance values, and according to the regression equation each per cent increase in the content of free iodine caused a decrease of 0.4 ml/min/kg in the clearance value.

Influence of carrier. As can be seen in Table I the paired experiments with and without carrier in the same animals showed no increase but rather indicated a decrease in the clearance values of both test substances when carrier was used. The decrease was, however, not statistically significant.

The clearance values in three patients given iodohippurate with carrier were 426, 452 and 333 ml/min/1.73 m², respectively, and did not differ markedly from those of the three patients investigated without carrier, being 407, 434 and 414 ml/min/1.73 m².

Uptake into red blood cells. In the in vitro experiments with iodohippurate (Fig 2) an equilibrium between plasma and red blood cells was reached after about 45 minutes, when about 74 % of the activity was in the plasma and 26 % in the red blood cells of dog blood, whereas the plasma and the red cells of human blood contained about 88 % and 12 % of the activity, respectively. The results were the same whether the initial movement of the test substance was from plasma to red blood cells (Type I experiments) or vice versa (Type II experiments). The initial rate of uptake was for dog blood 5.0 % per minute and for human blood 1.7 % per minute.

Table I. Influence of carrier on mean clearance values

Method	Number of experiments	Clearance		
		No carrier	Carrier	Difference ^a
<u>Iodohippurate</u>		ml/min/kg		
Single injection, plasma	9	9.84	9.15	-0.69 ± 1.46
Single injection, blood	12	12.82	12.47	-0.35 ± 2.70
Continuous infusion, plasma	9	8.81	8.83	+0.02 ± 2.35
Continuous infusion, blood	14	11.91	11.58	-0.33 ± 3.44
<u>Iothalamate</u>				
Single injection, plasma	6	3.18	2.93	-0.25 ± 0.66
Single injection, blood	6	5.19	4.49	-0.62 ± 1.04
Continuous infusion, plasma	6	3.18	2.95	-0.22 ± 0.54
Continuous infusion, blood	6	5.14	4.51	-0.52 ± 0.92

^a Mean and standard deviation

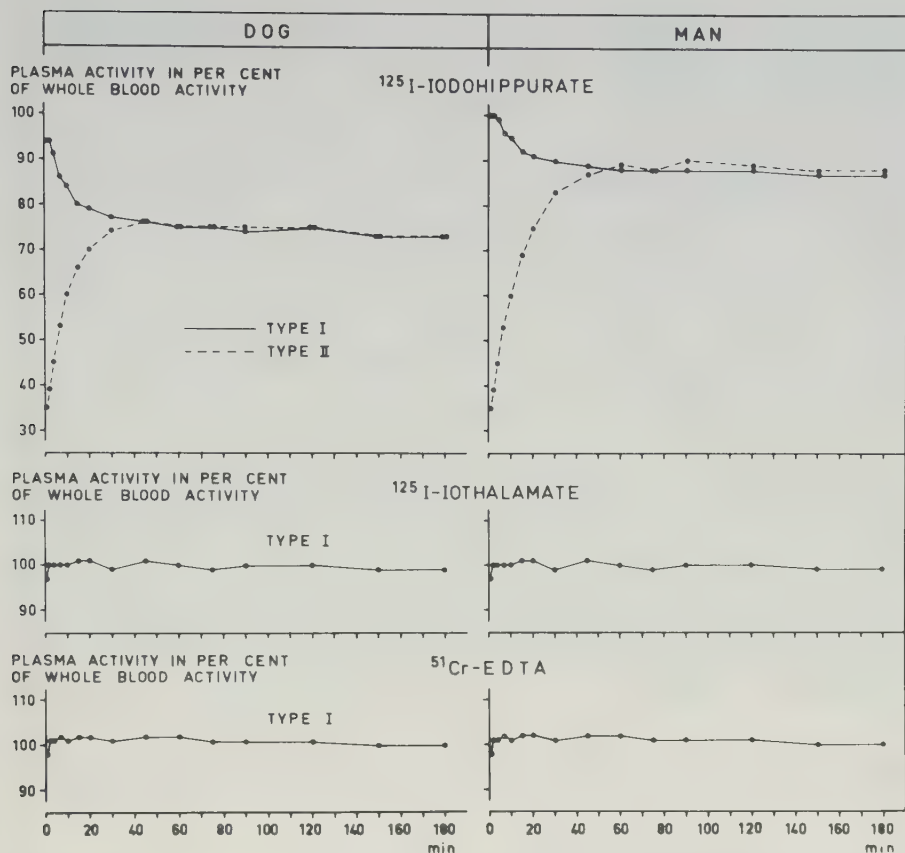


Fig 2 In vitro investigations of the uptake of the test substances into red blood cells. In type I and type II experiments the test substance is initially in plasma and in packed red cells, respectively.

iothalamate and Cr-EDTA did not show any red blood cell uptake in vitro, the activity in plasma remaining at about 100 % throughout the tests in dog blood as well as in human blood.

The in vivo experiments (Fig 3) disclosed that when given by continuous infusion 25 % of the iodohippurate was in the red blood cells of dog blood and 11 % in the red blood cells of human blood. When iodohippurate was given by single injection an equilibrium seemed established after about 15 minutes in the dog and after about 30 minutes in man, the red blood cells then containing on the average 35 % and 24 % of the activity, respectively.

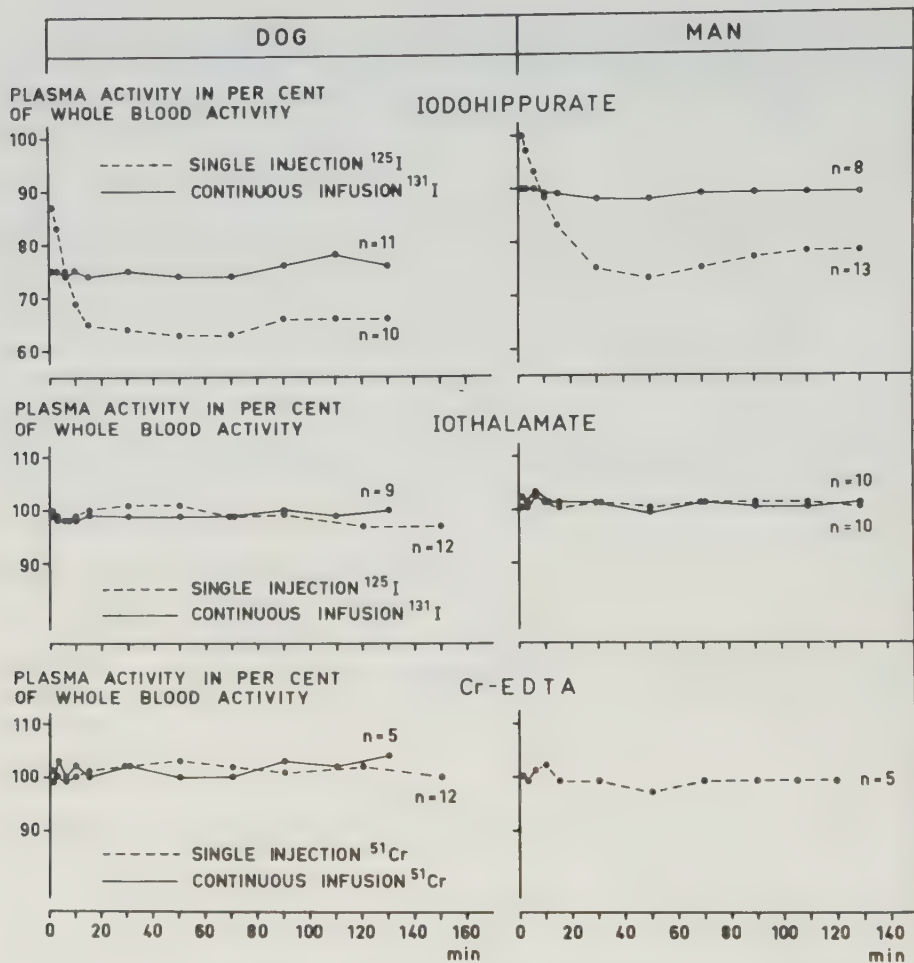


Fig 3 In vivo investigations of the uptake of the test substances into red blood cells.

For iothalamate and Cr-EDTA no red cell uptake could be demonstrated whether they were given by continuous infusion or by single injection. In the dog mean plasma values were 99 % for iothalamate and 101 % for Cr-EDTA, while the mean values in man were 101 % for iothalamate and 99 % for Cr-EDTA.

The four experiments with centrifugation of the blood at different times after drawing (Fig 4) showed that when the blood was drawn 1 or 3 mi-

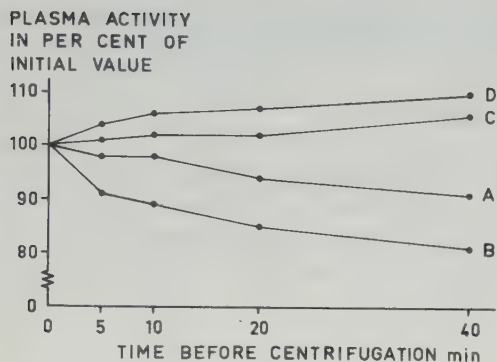


Fig 4 Influence of delayed centrifugation of the samples on the plasma activity values. Each line represents one experiment. The blood sample was drawn in A at 1 minute, in B at 3 minutes, in C at 10 minutes and in D at 30 minutes after the injection of the test substance. The time scale denotes length of delay before centrifugation.

minutes after the administration of the iodohippurate, this moved from plasma to cells. A delay of only five minutes before centrifugation gave a plasma value that was on the average 5 % too low while a delay of twenty minutes caused an error of 10 %. When the blood was drawn 10 minutes or later after the iodohippurate had been given, this moved from cells to plasma. In this case a delay of five minutes before centrifugation gave a plasma value that was on the average 2.5 % too high and a delay of twenty minutes caused an error of about 5 %.

The red blood cell uptake in arterial and venous blood of iodohippurate given as a single injection is shown in Fig 5. About 15 minutes after the

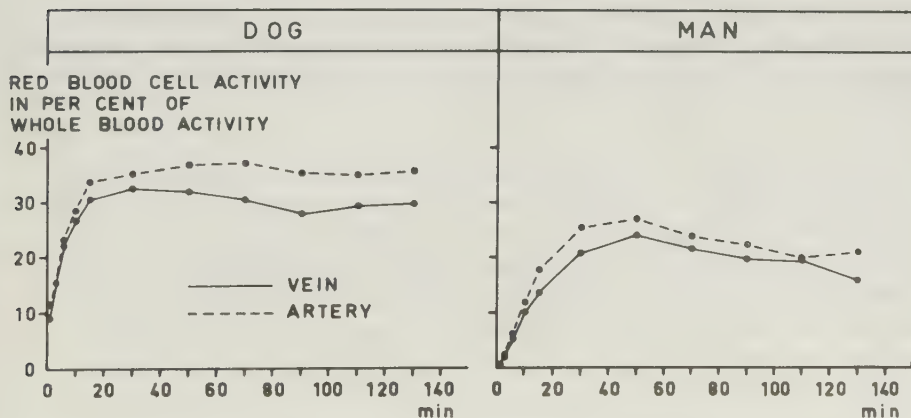


Fig 5 Uptake of iodohippurate into arterial and venous red blood cells after single injection.

administration of the iodohippurate the arterial red cells contained on the average 18 % more than the venous red cells in dog blood and 15 % more in human blood.

Resorption from the urinary bladder. Comparison of the plasma activities found during the tests concerning resorption from the bladder with those obtained in continuous infusion clearance experiments indicated that for both test substances less than 1 % of the activity instilled into the bladder was resorbed during one hour.

Arterio-venous difference. The mean V/A-quotients during the course of the experiments are depicted in Fig 6. When continuous infusion was used the quotients stayed around 1.0 for iodohippurate as well as for iothalamate, for the dog and for man, in plasma and in blood. When single injection was used the V/A-quotients in all cases started below 1.0, then at first very rapidly and later slowly increased to levels varying between 8 % and 40 % above 1.0, which was passed for iodohippurate at about 3 minutes and for iothalamate at about 10 minutes. The mean values for the three experiments with ^{51}Cr -EDTA in the dog did not differ from those of iothalamate.

The influence of the arterio-venous difference on the clearance values as calculated on arterial and venous plasma is accounted for in Table II. It is evident that for iodohippurate the venous clearance values were lower than the arterial ones in all periods and the difference increased during the first three or four periods, but remained constant during the last periods. The fourth period value was 77 % and 88 % of the first period value in the dog and in man, respectively. The venous clearance decreased progressively in relation to the arterial clearance when calculated for increasing period lengths.

For iothalamate the venous clearance was higher than the arterial during the first period but increasingly lower during the following three periods, and thereafter the relation between the two clearance values remained constant. The fourth period value was 90 % and 86 % of that of the first period in the dog and in man, respectively. The venous clearance was equal to the arterial at a periodlength of 40 minutes in the dog and 100 minutes in man.

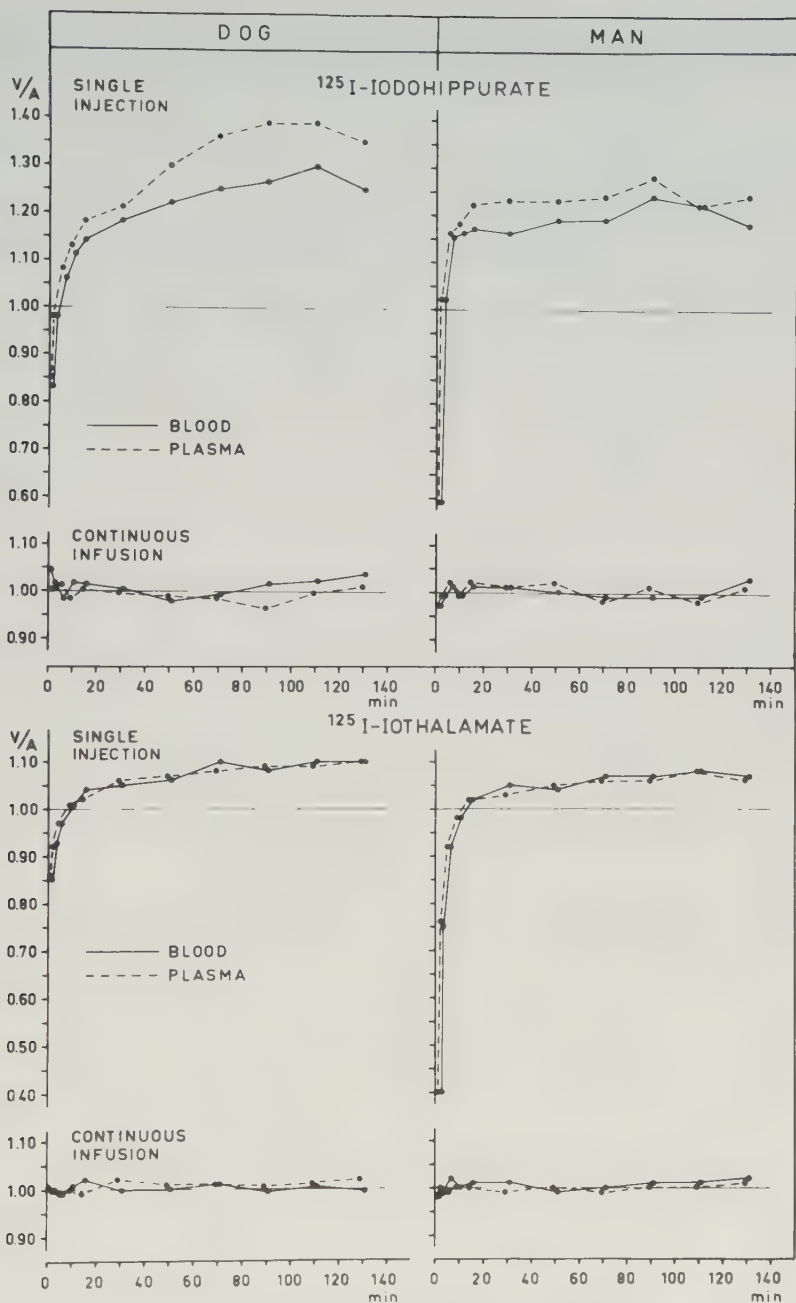


Fig 6 Arterio-venous differences of iodohippurate and of iothalamate after single injection and during continuous infusion in dogs and in man.

Table II. Quotients between venous and arterial clearance values

Clearance period	Dog				Man			
	Iodohippurate		Iothalamate		Iodohippurate		Iothalamate	
	Per period	Accumulated ^a	Per period	Accumulated	Per period	Accumulated	Per period	Accumulated
I	0.96	0.96	1.02	1.02	0.92	0.92	1.10	1.10
II	0.83	0.93	0.95	1.00	0.82	0.89	0.98	1.04
III	0.78	0.92	0.94	0.99	0.81	0.89	0.97	1.03
IV	0.74	0.90	0.92	0.98	0.81	0.88	0.95	1.01
V	0.73	0.89	0.92	0.97	0.81	0.88	0.94	1.00
VI	0.74	0.88	0.92	0.97	0.81	0.87	0.94	1.00

^a For increasing period lengths from 20 to 120 minutes.

Delay time. In Fig 7 are given the results of the delay time determination in three dogs. From about ten minutes after the administration of iothalamate and onwards the delay time was about two minutes.

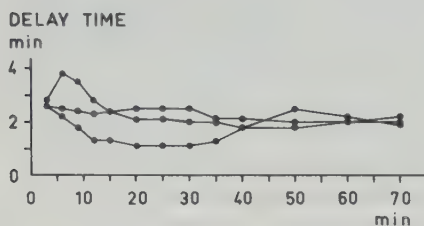


Fig 7 Results of the delay time determination in three dogs. The time was read off as shown in Fig 1.

DISCUSSION

Influence of free iodine. The clearance of free iodine is only about 5 - 10 % of that of iodohippurate (Giebisch, MacLeod & Kavalier, 1956) and therefore the relative content of free iodine in plasma will increase with time. This probably explains why an influence of free iodine could be demonstrated in the continuous infusion series but not in the single injection series. In the latter type of experiments the clearance measurements start at once after the test substance has been given, while in the former type of experiments the continuous infusion has been going on for at least one hour before the clearance determinations begin.

Meschan, Hosick, Schmid & Watts (1963) found that each per cent free iodine caused a depression of the clearance values, amounting to on the average 4.7 ml/min, which corresponds fairly well with the present result of 0.4 ml/min/kg.

That the presence of free iodine in a test substance labelled with radioactive iodine has an influence on the clearance value has been pointed out by several authors, but the opinions about the degree of influence differ widely. Burbank, Tauxe, Maher & Hunt (1961) state that more than 2 % free iodine is not acceptable, but Schwartz & Madeloff (1962) consider 3 % to be without influence and Meschan, Schmid, Watts & Witcofski (1963) feel that up to 10 % can be accepted. Bianchi, Hegesippe, Meozzi, Rosa & Sosi (1965) found that even moderate amounts of free iodine had a considerable effect on the clearance values. In their calculations, however, they did not take the thyroid uptake into consideration and in their experiments they ascribed the total fall in the clearance values to the presence

of free iodine, which they considered as the only reason for the decrease. There are, however, also other possible reasons as shown in the present study and in a following investigation (Pihl, 1973).

Influence of carrier. It is well known that protein-binding of iodopyracet and vitamin B₁₂ causes a depression of their clearance when these substances are given in tracer doses and that the prior addition of non-radioactive carrier can prevent this depression by saturation of the binding sites (Block & Burrows 1958; 1960; Nelp, Wagner & Reba 1964; Ekins, Nashat, Portal & Sgherzi 1966). Very divergent results have been published about the protein-binding of iodohippurate. Smith & Smith (1938) found 70 % to be bound in man and 78 % in the dog, while Magnusson (1962) found practically no binding in man. Kimbel (1963) stated that 40 to 60 % was proteinbound, dependent upon the concentration. The present results with the use of nonradioactive carrier iodohippurate indicate that no protein-binding of the radioactive moiety that is of importance for the clearance values occurs. This is in accord with the results of Maher, Tauxe, Strong & Elveback (1970) and Pixberg, Hilger, Zielske, Bahlmann & Gollnisch (1971) who found that the addition of carrier iodohippurate had very little, if any, influence on the clearance values of radioactive iodohippurate.

The addition of carrier iothalamate had no elevating effect on the clearance values of radioactive iothalamate, which was also found by Sigman, Elwood, Reagan, Morris & Cantanzaro (1965), Anderson, Sawyer & Cutler (1968) and Gagnon, Schrier, Weis, Kokotis & Mailloux (1971).

Uptake into red blood cells. There was a substantial uptake of iodohippurate into the red cells of dog blood, and although the uptake into human red blood cells was less pronounced it was still considerable. For the dog as well as for man the results of the in vitro experiments agree very closely with those in vivo experiments where iodohippurate was given as a continuous infusion, the uptake into the red blood cells being practically the same. When iodohippurate was given as a single injection, however, the fraction of the total blood content of iodohippurate retained in the red blood cells was much larger than when given as a continuous infusion. This obviously depends on the fact that iodohippurate is extracted from the plasma by the kidneys and since the attainment of equilibrium between plasma and cells is a relatively slow process, a gradient must exist between the actual level in the red blood cells and the level at which they are in equilibrium with the plasma.

The investigation of red cell uptake in simultaneously drawn arterial and venous blood was prompted by the finding of a difference in V/A-quotients between whole blood and plasma. A higher uptake in the arterial red cells than in the venous cells of dog blood as well as of human blood was disclosed, and this explains the difference in the V/A-quotients between whole blood and plasma. The difference in red cell uptake between arterial and venous blood can be explained by the fact that the arterial blood corresponds in its composition to the blood in the right atrium; a considerable part of this blood was immediately before arrival to the heart deprived of most of its plasma content of iodohippurate by the action of the kidneys. The ratio between the concentration in red cells and in plasma must therefore be larger in arterial blood than in venous blood, which has been allowed more time to equalize the concentration difference between red cells and plasma.

Smith, Finkelstein, Aliminosa, Crawford & Graber (1945) found a red blood cell/plasma distribution ratio in vivo for non-radioactive iodohippurate of 0.65 for the dog and 0.49 for man. Our in vivo results expressed the same way are 0.44 - 0.72 for the dog and 0.16 - 0.42 for man.

Burbank et al. (1961) and Magnusson (1962) used ^{131}I -iodohippurate and found a red blood cell/plasma distribution ratio for man in vivo of 0.29 and 0.43, respectively. Blum & Scholz (1963) found considerably higher values for the cell uptake of ^{131}I -iodohippurate in human red blood cells suspended in an electrolyte solution.

Burbank et al. found the rate of uptake to be 5.5 % per minute and Magnusson found 8 % per minute for man. These rates are considerably higher than the rate of 1.7 % per minute found in the present investigation, but since neither of the above-mentioned authors state their method of calculation, direct comparisons cannot be made.

The results of the experiments with centrifugation of the blood at different times after drawing illustrate the importance of immediate centrifugation of the samples, delay giving errors of up to 12 %.

Iothalamate and Cr-EDTA was not taken up by the red cells of dog blood, nor by those of human blood and this is in agreement with earlier reports (Garnett, Parsons & Veall, 1967; Anderson et al. 1968; Pixberg, Hilger & Zielske, 1969).

Resorption from the urinary bladder. Heidenreich, May & Kook (1961) found considerable resorption of PAH (para-aminohippuric acid) from the bladder of dogs, and Maluf (1955) reported Urokon (sodium acetrizoate)

to be resorbed from the human bladder. In the present investigation the resorption from the bladder during one hour was found to be less than one per cent of the activity in the bladder even when the amount excreted during the experiment was taken into consideration. This agrees with findings reported by Marucci, Shoemaker, Wase, Strauss & Geyer (1954) and by Englund (1956).

Arterio-venous difference. Immediately after a single injection of a clearance substance the concentration of the substance is higher in the arterial than in the venous blood, but very soon the curves cross - the concentrations being equal for one moment only - and at all later times the venous blood shows the higher concentration. Brun, Hilden & Raaschou (1949) pointed out that the magnitude of the difference between arterial and venous blood depends on the clearance. For iodohippurate the arterio-venous difference is considerable, especially in the dog but also in man. That the difference is more pronounced for plasma than for whole blood depends, as stated above, on the fact that the arterial red blood cells contain more iodohippurate than the venous cells. Iothalamate displayed a much smaller arterio-venous difference than iodohippurate, and this was to be expected because of its lower clearance. Blood and plasma did not differ from each other, which is in accord with the absence of red blood cell uptake of this substance.

The present results are in line with those of earlier reports. Brun et al. (1949) found a mean arterio-venous difference of 29 % for diodrast and 7 % for inulin. Dutz (1952) found 22 - 26 % for PAH, and Slot (1965) found about 9 % for exogenous creatinine. Truninger, Donath & Kappeler (1968) found 30 % for iodohippurate and 11 % for Cr-EDTA.

The influence of the arterio-venous difference on the clearance values was considerable; for iodohippurate as well as for iothalamate the venous clearance values decreased progressively during the first four periods. The decrease was more pronounced for iodohippurate than for iothalamate in the dog, but in man it was about equal for both test substances. For iothalamate in the dog and for both test substances in man the decrease amounted to 10 to 14 %, but for iodohippurate in the dog it was 23 %.

Venous clearance values were also calculated for increasing period lengths to find out to what extent late low values could be compensated by early high values. For iothalamate these clearance values became equal to the arterial values at 40 minutes in the dog and at 100 minutes in man. For iodohippurate full compensation could not be attained.

Delay time. The delay time correction most often used seems to be two and a half minutes as recommended by Smith (1951). This was based on investigations in man. A delay time of two minutes in dogs as found in the present investigations is in agreement with results in other studies. Morales, Crowder, Fishman, Maxwell & Gomez (1950) and Ekins, Nashat, Portal & Sgherzi (1966) found 100 seconds and two and a half minutes, respectively, using quite different methods.

In conclusion, two of the factors investigated, namely the arteriovenous difference and the presence of free idoine in the test substance, seem to be of importance for the decrease in clearance values found in an earlier investigation (Pihl & Nosslin, 1973). The decrease in the iodohippurate clearance values after single injection in this study amounted to about 40 %, and the present investigation shows that a decrease of 23 % can be explained by the arterio-venous difference. The effect of free iodine seems to be limited since in the case of single injection no significant correlation could be demonstrated between the free iodine content and the clearance values. This question, together with other possible explanations for the decreasing clearance, will be dealt with in further studies.

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The single injection technique for determination of renal clearance III.

Renal extraction of iothalamate, iodohippurate and Cr-EDTA.

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The present investigation of the renal extraction rate of some radioactive clearance substances was prompted by an earlier investigation of the canine renal clearance of two of these substances (Pihl & Nosslin, 1973). In this study a considerable fall in the clearance values from period to period for iodohippurate given as a single injection was found, and a decreasing extraction was considered as one of the possible explanations. No previous systematic studies of the renal extraction after single injection seem to have been published.

MATERIAL AND METHODS

Animals. Thirteen female, mongrel dogs weighing 12 - 25 kgs were used. They were kept on the ordinary diet of the laboratory. During at least twelve hours before each experiment they were given no food but allowed water ad libitum.

Patients. Fourteen patients of both sexes varying in age between 24 and 77 years underwent determination of the renal extraction in connection with renal angiography at the Department of Diagnostic Radiology. No patient had severely reduced kidney function. Serum creatinine was within 0.6 - 1.2 mg/100 ml.

Test substances. Sodium iothalamate and sodium ortho-iodohippurate labelled with ^{125}I or ^{131}I and Cr-EDTA labelled with ^{51}Cr were purchased from the Radiochemical Centre, Amersham, England. After appropriate dilution they were kept in the dark in the refrigerator. Substances labelled with ^{131}I were never kept for more than two weeks. Each batch of the iodine-labelled test substances was checked for free iodine by chromatography as earlier described (Pihl, 1973). For every experiment 50 - 100 μCi (in the case of Cr-EDTA up to 300 μCi) of the test substances were used, corresponding to 0.5 - 20 mg of iothalamate, 0.25 - 10 mg of iodohippurate and less than 1 mg of Cr-EDTA. The single injection dose, the priming dose and the dose standard were made up as earlier described (Pihl & Nosslin, 1973).

Experimental procedure. The extraction and clearance determinations in dogs were carried out under general anesthesia with endotracheal intubation. In Series I the anesthesia was induced with dilute sodium

pentobarbital 25 mg per kg bodyweight and maintained with small supplementary doses as needed. The induction was slow and watchful. The ventilation was maintained with a constant volume respirator. In Series II the anesthesia was induced with sodium thiopental 20 mg per kg bodyweight and maintained with a mixture of N_2O and O_2 (4:1) combined with suxamethonium chloride as a muscle relaxant. A constant volume respirator was used and the ventilation was controlled by keeping the alveolar air concentration of CO_2 constant at 5 % with the aid of a Godart capnograph CG 11900. In a group of 13 dogs, where extraction only was measured, ordinary sodium pentobarbital anesthesia without respirator was used.

The animals were tied in a supine position on an animal board and a bladder catheter (usually Charrière 14) was applied with the help of a speculum and fixed with adhesive tape. Control samples of urine and blood were taken and 400 - 500 ml of physiologic saline with 5 % mannitol was administered intravenously. After injection of the priming dose the continuous infusion, also containing 5 % mannitol, was started, being given by an infusion pump at a rate of 2.8 ml/min. When both substances were given by single injection, saline with 5 % mannitol was given as continuous infusion.

With the Seldinger technique red Ödman-Ledin catheters No 1 (OD 2.2 mm, ID 1.2 mm) were placed in the femoral artery and vein. The distal end of the venous catheter was preformed to facilitate entrance of the renal vein and was provided with two extra side holes within one cm of the tip. Under fluoroscopic control the tip of the venous catheter was manipulated into the right renal vein and the tip of the arterial catheter was placed in the aorta at the level of the renal arteries. One hour or more after the beginning of the continuous infusion, the urinary bladder was emptied, rinsed with saline and air, and the single injection was given. At 1, 3, 6, 10, 15, 30, 50, 70, 90, 110 and 130 minutes blood samples of 5 ml were drawn into heparinized syringes from the arterial and venous catheter simultaneously, care being taken to discard the content of the catheter each time. To avoid admixture from the vena cava some 20 - 30 seconds were allowed for the drawing of the venous sample and the arterial sample was taken in the middle of this period. Centrifugation of the samples at 3800 rpm during five minutes was done immediately and the plasma was pipetted off at once. The samples were divided before centrifugation so that blood as well as plasma could be measured. The total blood loss for the animal during one experiment was about 130 ml corresponding to a mean plasma 'clearance' of 0.04 ml/min/kg. In the 13 dogs where extraction was determined without concomitant clearance measurement only whole blood was used and the samples were 2 ml.

Six urine collection periods of 20 minutes each were run, the bladder being flushed with 50 ml of saline and then repeatedly with air at the end of each period. Urine and irrigating fluid were combined after the volumes had been measured. At the end of the experiment 1 ml aliquots of all blood, plasma and urine samples were taken by constriction pipettes into special plastic tubes for counting. Hematocrit was determined at the beginning and at the end of the experiment.

Patients that were to have a renal angiography were given about 500 ml of water orally in the morning and were kept in bed. An intravenous cannula was placed in an arm vein and a control blood sample was obtained. The single injection dose, the priming dose and the continuous infusion were given through this cannula. The continuous infusion fluid was physiologic saline, which was administered by an infusion pump at a rate of 0.5 ml per minute. The patient was given 300 ml of water orally per hour. Under local anesthesia, red Ödman-Ledin catheters No 7 (OD 2.2 mm, ID 1.4 mm) were placed in the right renal vein and in the aorta in the manner described above for dogs. One hour after the start of the continuous infusion the single injection was given and blood samples were then taken simultaneously from the arterial and venous catheters with the same timing and in the same way as described for dogs. If both test substances were given by continuous infusion the sampling started one hour after the beginning of the continuous infusion. If both test substances were given by single injection, saline only was given as continuous infusion. The samples were treated as described for dogs. The angiography was performed after completion of the extraction studies.

Series. Two series of dogs were run. Four experiments had to be discarded due to various technical reasons. In Series I which consisted of 15 experiments ^{125}I -iothalamate was given as a single injection and ^{131}I -iothalamate as a continuous infusion. In six of the experiments carrier in the form of Conray[®] containing 0.5 g sodium iothalamate per ml was used. Two ml were given as a priming dose and eight ml were added to the infusion fluid (1000 ml) to give calculated plasma levels of 20 - 30 mg%. In Series II were 17 experiments with ^{125}I -iodohippurate given as a single injection and ^{131}I -iodohippurate (12 experiments) or ^{51}Cr -EDTA (5 experiments) given as a continuous infusion. In four experiments carrier iodohippurate in the form of commercial ortho-iodohippuric acid (Hopkin & Williams, Ltd., England) prepared for intravenous use by the hospital pharmacy, was used. The priming dose was 0.1 - 0.2 g and 1.0 - 1.5 g was added to the infusion fluid (1000 ml) to give calculated plasma levels of 2 - 3 mg%.

In a group of 13 dogs ^{125}I -iothalamate and ^{51}Cr -EDTA were both given by single injection. In two dogs two single injections were given, the second about two hours after the first one. The extraction was determined immediately after the first injection and just before and immediately after the second injection. One dog was given ^{125}I -iodohippurate and ^{51}Cr -EDTA in both injections and the other dog received ^{125}I -iodohippurate and ^{131}I -iothalamate in both injections.

Two series of patients were investigated. In Series A nine patients were given ^{125}I -iodohippurate and ^{51}Cr -EDTA, both as a single injection. In four cases carrier iodohippurate was given as a continuous infusion. The priming dose was 0.4 - 0.6 g and 2.4 g was added to the infusion fluid (200 ml) to give plasma levels of about 2.5 mg%. Series B comprised five patients given ^{125}I -iodohippurate and ^{131}I -iothalamate both as a continuous infusion.

Measurement of radioactivity. The radioactivity was measured in 1 ml samples of plasma or blood and urine in a Packard automatic gamma spectrometer model 5212 using standard dual-channel technique, as earlier described (Pihl & Nosslin, 1973). The window setting was the same for ^{131}I and ^{51}Cr . A fixed counting time of 10 minutes was used. The maximum counting errors as calculated according to formulas given by Ambrosen (1967) were in the animal experiments for iothalamate whether given as a single injection or as a continuous infusion and whether measured in plasma or in blood less than 2.5 %. For Cr-EDTA the error was not over 5 % with one exception where it reached 10 %. The error for iodohippurate in continuous infusion was not over 5 % whether in plasma or in blood. After single injection the error for iodohippurate was below 8 %, except in one case where it reached 12 %. In the human investigations the error for iothalamate and for Cr-EDTA never exceeded 4 %. For single injection of iodohippurate the error was 7 % and for continuous infusion 11 %. The errors given are maximum errors obtained on measurement of the last venous samples.

Calculations. The extraction percentage was calculated according to the formula:

$$\text{Ex} = \frac{(c_A - c_V) \times 100}{c_A}$$

where c_A and c_V are given as cpm per ml arterial and venous plasma or blood, respectively.

In order to find out the influence on the extraction values of any shift of activity in the blood between red blood cells and plasma during the time from the passage of the kidney until the plasma was separated after centrifugation, a theoretical extraction value was calculated. The measured plasma (P_A) and whole blood (B_A) values of arterial blood, the measured renal venous whole blood value (B_V), and the hematocrit, expressed as a fraction (Hct), were used to calculate a theoretical value for renal venous plasma (P_V). Under the assumption that no substance is extracted directly from the red blood cells and that no shift occurs between red cells and plasma during or after the passage of the kidney, whether in vivo or in vitro, the total cpm of a given volume of red blood cells must be the same in renal venous blood as in arterial blood. The cpm of arterial red cells in 1 ml blood is $B_A - (1 - \text{Hct}) P_A$ and, assuming the cpm of renal venous blood cells to be the same, the activity of plasma in 1 ml of renal venous blood is $B_V - (B_A - [1 - \text{Hct}] P_A)$. Using this theoretical P_V a formula for a theoretical extraction percentage can be written:

$$\frac{\text{Ex}}{100} = \frac{(1 - \text{Hct}) P_A - B_V + (B_A - [1 - \text{Hct}] P_A)}{(1 - \text{Hct}) P_A}$$

which can be simplified to:

$$\text{Ex} = \frac{(B_A - B_V) \times 100}{(1 - \text{Hct}) P_A}$$

This formula can also be derived more directly by assuming that the changes in the whole blood activity are due to changes in plasma activity only.

The uptake into red blood cells in vivo was calculated as earlier described (Pihl, 1973).

The renal circulation was calculated according to the formula of Wolf (1941):

$$\text{Circulation} = \frac{V \times (U - c_V)}{c_A - c_V}$$

where V = urine flow in ml per minute, U = cpm per ml urine, c_V = cpm in 1 ml renal venous plasma or blood and c_A = cpm in 1 ml arterial plasma or blood.

The continuous infusion clearance was calculated according to the standard formula:

$$C = \frac{U \times V}{P}$$

where U = cpm per ml urine, P = cpm per ml plasma or blood, and V = urine flow in ml per minute. The single injection clearance was calculated according to the formula:

$$C = \frac{E}{A}$$

where E = total amount excreted in urine during a certain time, and A = area under the plasma curve during the corresponding time period (Pihl & Nosslin, 1973). A delay time correction of two minutes was used (Pihl, 1973).

Statistical methods. Statistical comparisons were based on Student's t-test for paired observations and on analysis of variance for groups of observations. Regression within groups was studied with analysis of covariance, otherwise standard statistical procedures were used (Snedecor, 1955). When calculating the regression for extraction values all values before ten minutes were omitted. Regression expressed as per cent change per period was obtained by multiplying the regression coefficient by period length x 100 and dividing by the mean extraction or clearance value. Differences found were considered probably significant if $P < 0.5$ and significant if $P < 0.01$.

RESULTS

The content of free iodine was in all cases below 1 % (mean 0.7 %) for ^{125}I and below 2.5 % (mean 2.0 %) for ^{131}I . No significant influence of free iodine on the extraction values could be demonstrated.

Mean values and regressions for extraction and clearance in dogs are given in Table I.

Iothalamate and Cr-EDTA. No significant difference was found between the six experiments in Series I where carrier iothalamate was given and the rest of the experiments in this series. The circulation as calculated

from the continuous infusion values showed no significant change with time.

The results from Series I are depicted in Fig 1, which shows that the extraction of iothalamate in dogs was practically the same whether the substance was given as a single injection or as a continuous infusion; the correlation coefficient was 0.98. The mean extraction for ^{125}I -iothalamate given as a single injection was 22.2 % when measured on plasma and 23.3 % when measured on whole blood. Corresponding figures for ^{131}I -iothalamate given as a continuous infusion were 22.8 % and 23.2 %, respectively. The differences between the single injection values and the continuous infusion values and between the plasma and the blood values were not significant. From the time curve in Fig 1 it is evident that the extraction values did not change with time and the regression coefficients were not significantly different from zero.

The comparison of the extraction of ^{125}I -iothalamate and ^{51}Cr -EDTA when given as a single injection in 13 dogs gave the results shown in Fig 2. The time curves for the extraction run practically parallel, the Cr-EDTA curve lying somewhat higher than that of iothalamate. The mean extraction for ^{51}Cr -EDTA was 27.4 % and that of ^{125}I -iothalamate was 26.6 %, the correlation coefficient being 0.84. The difference was not significant. The time curves showed a tendency to

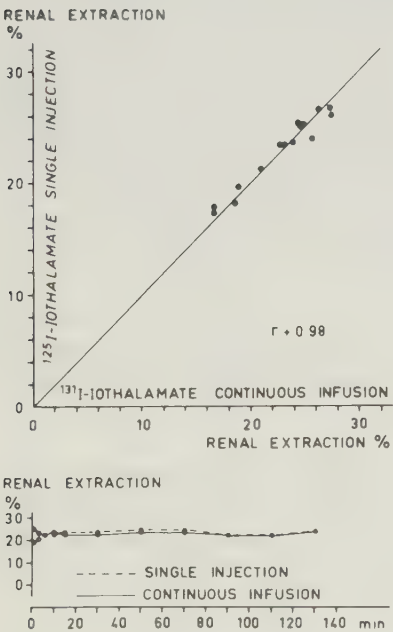


Fig 1. Correlation between simultaneous determinations of renal extraction of iothalamate given as single injection and continuous infusion in 15 dogs (Series I). Line of identity inserted. In lower part of figure the mean time curves are shown.

rise which was significant. Fig 3 shows the results when two single injections, the second two hours after the first, were given to two dogs. It is evident that neither the extraction of iothalamate nor that of Cr-EDTA changed during the experiment.

The results of the investigations in patients are given in Figs 4 and 5 where the extraction of ^{51}Cr -EDTA given as a single injection as well as that of ^{131}I -iothalamate given as a continuous infusion is seen to remain essentially constant with time. The regression coefficients were not significantly different from zero. The mean extraction value for ^{51}Cr -EDTA was 17.2 % and the range was 15.7 - 20.4. Corresponding figures for ^{131}I -iothalamate were 16.7 % and 11.7 - 20.1.

Iodohippurate. When carrier iodohippurate was used in dogs the mean extraction was clearly lower than when no carrier was given and the difference was significant (Table I).

The extraction of iodohippurate given as a single injection and as a continuous infusion in dogs is shown in Fig 6. For continuous infusion the mean extraction was 75 % in plasma and 59 % in blood. After single injection of the test substance the extraction started at 87 % for plasma and 83 % for blood and fell continuously with time to 62 % and 46 %, respectively, after two hours. There was also a slight but significant fall with time in the extraction values when iodohippurate was given as a continuous infusion.

The distribution of activity between red blood cells and plasma in arterial and renal venous blood after single injection is depicted in Fig 7, which shows that in the arterial blood the distribution of activity between plasma and cells is close to the in vitro equilibrium at all times. In renal venous blood, on the other hand, the distribution is quite different and far off from the equilibrium value.

The theoretical extraction values were found to be nearly the same as the measured values in the dog as well as in man irrespective of mode of administration. The results for single injection in the dog are shown in Fig 8.

The extraction of iodohippurate in two dogs given a second single injection dose two hours after the first one is shown in Fig 3. Immediately after the first injection the extraction was nearly 90 % but after two hours it was only about 60 %. Immediately after a new injection it was again about 90 %.

Table I. Results of extraction and clearance measurements in dogs

Test substance and Method	Number of experi- ments	Extraction				Clearance			
		Mean value $\pm$ SD		Regression (change/period ^a)		Mean value		Regression (change/period ^a)	
		Plasma	Blood	Plasma	Blood	Plasma	Blood	Plasma	Blood
		%		%		ml/min/kg		%	
Series I									
131I-iothalamate. Continuous infusion.	15	22.8 $\pm$ 0.9	23.2 $\pm$ 0.7	+ 0.4 ⁻	+ 0.2 ⁻	2.85	4.54	+ 1.3**	+ 1.7**
125I-iothalamate. Single injection.	15	22.2 $\pm$ 1.6	23.3 $\pm$ 1.1	+ 0.8 ⁻	+ 0.2 ⁻	2.82	4.50	+ 1.3**	+ 1.6**
Series II									
51Cr-EDTA. Continuous infusion.	5	22.8 $\pm$ 1.4	24.8 $\pm$ 1.8	+ 1.9 ⁻	+ 2.9**	3.52	5.97	+ 1.9**	+ 2.8**
131I-iodohippurate + Carrier. Continuous infusion.	4	70.0 $\pm$ 0.8	53.8 $\pm$ 1.2	- 0.3 ⁻	- 0.7 ⁻	8.58	11.47	+ 0.5 ⁻	+ 0.5 ⁻
131I-iodohippurate. Continuous infusion.	8	74.7 $\pm$ 1.1	59.2 $\pm$ 1.7	- 0.5**	- 1.3**	8.93	11.65	+ 0.3 ⁻	+ 0.9 ⁻
125I-iodohippurate. Single injection.	13	86.8 $\pm$ 4.5	83.0 $\pm$ 2.2	- 4.4**	- 4.7**	10.32	14.50	- 3.5**	- 5.8**
Last value		62.0 $\pm$ 7.5	45.6 $\pm$ 5.5			8.57	10.33		

^a Significance denoted by - for $P > 0.05$, * for $P < 0.05$ and ** for $P < 0.01$.

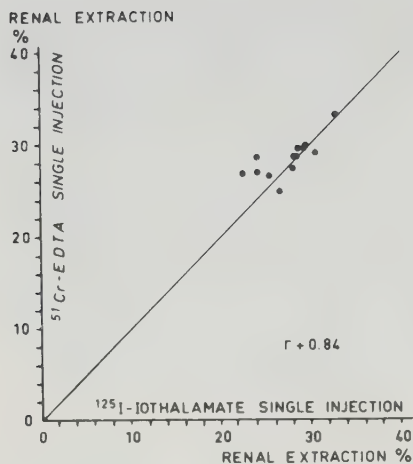


Fig 2 Correlation between simultaneous determinations of renal extraction of iothalamate and Cr-EDTA, both given as single injections in 13 dogs. Line of identity inserted. In lower part of figure the mean time curves are shown.

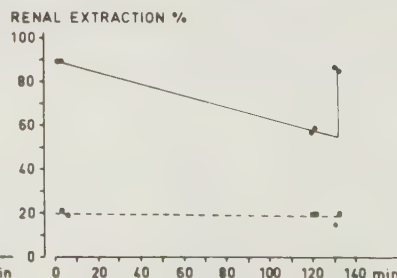
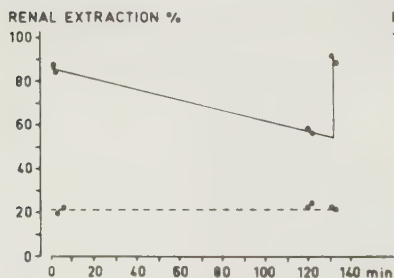
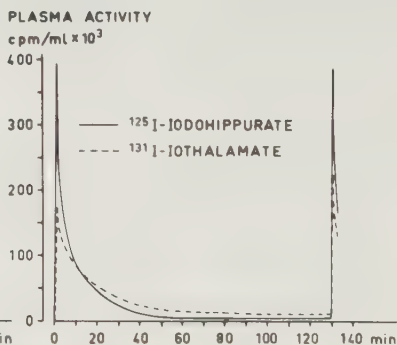
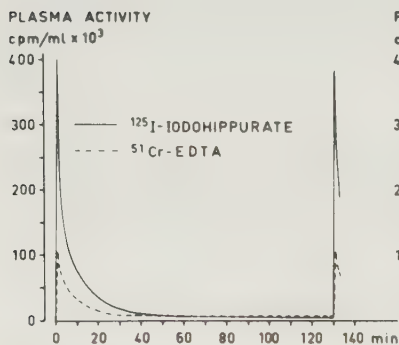
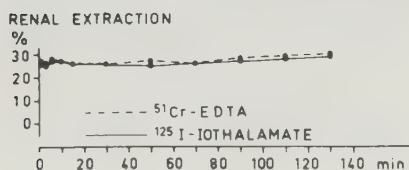


Fig 3 Plasma curves and renal extraction of iothiopyridate, iothalamate and Cr-EDTA in two dogs given two single injections at 0 and 130 minutes.

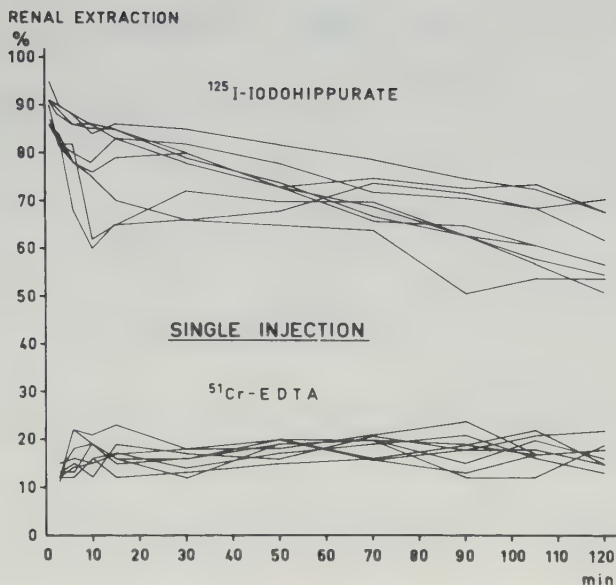


Fig 4 Renal extraction of iodohippurate and Cr-EDTA after single injection in 9 patients (Series A).

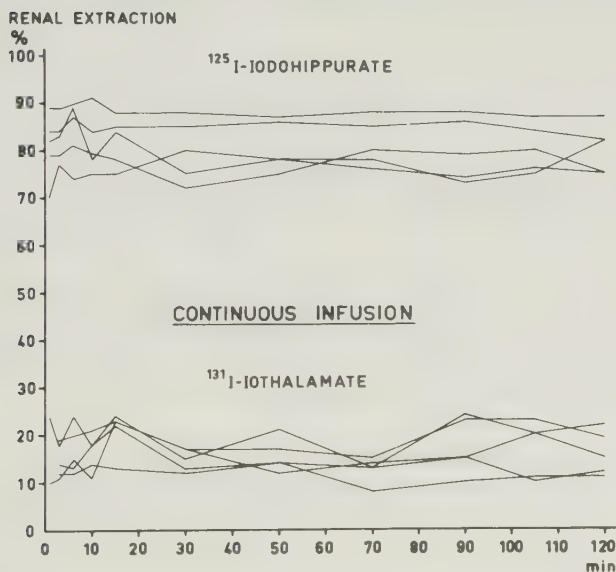


Fig 5 Renal extraction of iodohippurate and iothalamate during continuous infusion in 5 patients (Series B).

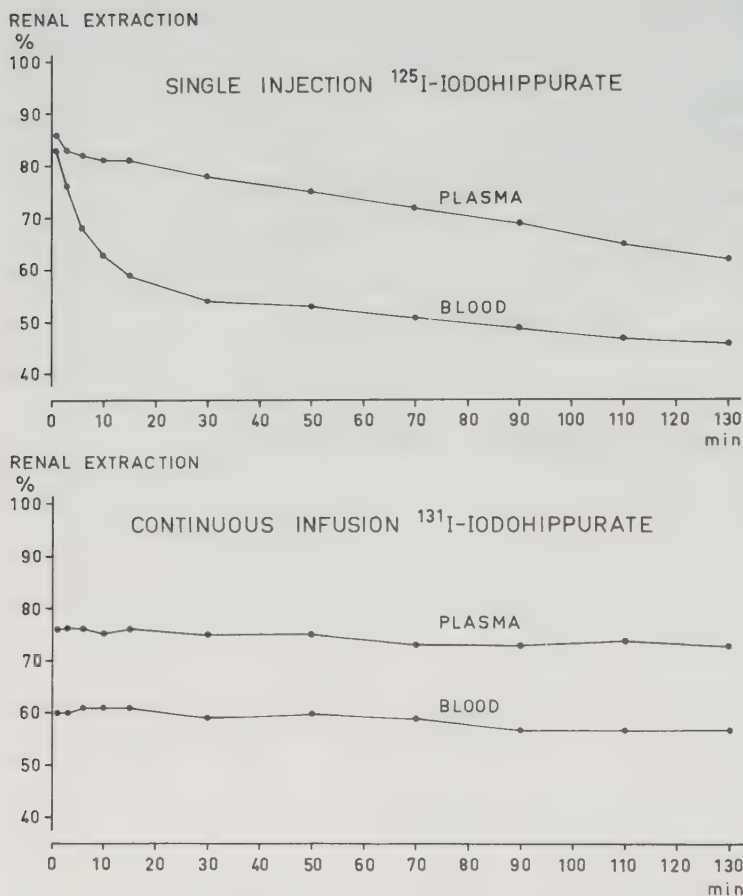


Fig 6 Renal extraction in plasma and blood of iodohippurate given as single injection (13 dogs) and as continuous infusion (8 dogs).

The continuous infusion clearance values were constant with time, and the circulation as calculated from the continuous infusion values did not show any significant change. After single injection the clearance values decreased with about 17 % in plasma and 29 % in blood.

In the investigations on patients (Figs 4 and 5) continuous infusion of iodohippurate was found to give a constant extraction with a mean value of 80 % and a range of 73 - 85 %. Corresponding figures for blood were 70 % and 68 - 74 %, respectively. After a single injection the extraction fell significantly with time, the decrease being 4.3 % per period for plasma and 4.8 % per period for blood. No influence of carrier iodohippurate could be demonstrated in man.

IODOHIPPURATE SINGLE INJECTION DOG

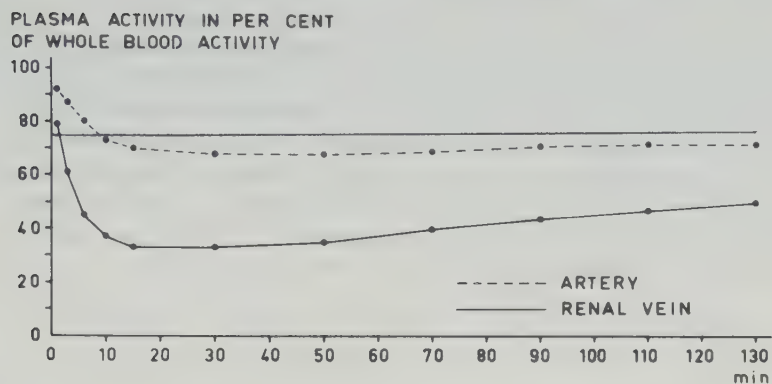


Fig 7 Distribution of iodohippurate between red cells and plasma in arterial blood and in renal venous blood. Inserted line denotes equilibrium value found in vitro.

IODOHIPPURATE SINGLE INJECTION DOG

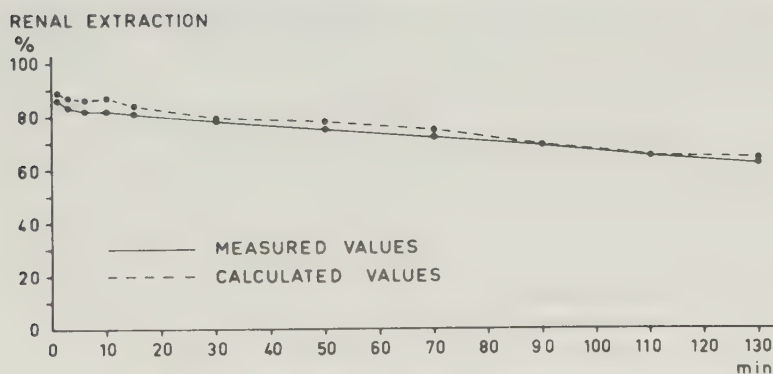


Fig 8 Calculated theoretical extraction values (see text) and measured extraction values after single injection of iodohippurate.

DISCUSSION

In order to avoid displacement of the arterial and renal venous catheters the animal experiments were performed under general anesthesia, although it is known that this may have a depressing action on the renal function (de Bodo & Prescott, 1945; Surtshin, Mueller & White, 1952; Blake, 1957). Also suxamethonium chloride has been found to influence the renal function (Granberg & Wåhlin, 1962; Grängsjö & Persson, 1971). In the present experiments, however, the animals acted as their own controls and the influence of the anesthesia was therefore of minor importance.

The renal extraction was always determined in the right kidney since on this side the renal vein usually does not receive the blood from the gonads. In normal cases there is no difference in extraction between the right and the left kidney in dogs (Åsheim, Helander & Persson, 1958) nor in man (Bucht, 1951). Since normal, healthy dogs were used the right kidney was considered representative for both sides.

Investigations by Lindell & Ohlin (1956), Helander, Persson & Åsheim (1958) and Leiter (1965) have shown that if catheterization of the renal artery is carefully performed there are no effects on the function of the kidney except during the first minutes. There seems to be no reason to assume any harmful effects on the kidney from the renal venous catheter.

The extraction of iothalamate was practically the same whether given as a single injection or as a continuous infusion and there was no change with time. The level of about 23 % in dogs seems somewhat low compared with reported extraction values of 30 % and 37 % (Abe, 1971; Gagnon, Schrier, Weis, Kokotis & Mailloux, 1971) but in connection with saline diuresis a mean extraction of 24 % has also been reported (Blantz, Katz, Rector & Seldin, 1971). The average value of 17 % in patients is close to the reported normal value of 19 % for the filtration fraction in man (Smith, 1956). The addition of carrier had no effect on the extraction values and no signs of red blood cell uptake were found. The comparison between iothalamate and Cr-EDTA showed them to behave in the same manner and the extraction of Cr-EDTA was not significantly different from that of iothalamate. An initial and transient depressing action on the kidney has been ascribed to sodium pentobarbital (Surtshin et al., 1952; Blake, 1957), and the increase in the extraction values with time probably depended on the anesthesia, which was less rigidly controlled in these experiments than in the remaining experiments in the present investigation. The constancy of the extraction of iothalamate and Cr-EDTA was further documented by the double single injection test. The investigations in patients also showed the level of extraction to be constant for both substances.

The slight decrease with time of the extraction of iodohippurate given as a continuous infusion in dogs could be due to the presence of free ^{131}I although no such influence of significance could be demonstrated. In an earlier investigation (Pihl, 1973) an influence of free iodine on the continuous infusion clearance values in dogs was found. Another possible explanation is the saline infusion, since it has been repeatedly found that saline infusion can cause an increase of the renal blood flow and a decrease of the extraction of para-aminohippuric acid (Harth, Kreienberg & Lutz, 1959, Early & Friedler, 1965; Bovee & Webster, 1971). A similar action has been ascribed to mannitol (Braun & Lilienfield, 1963). Harth et al. also found decreasing extraction values when the hematocrit decreased but in the present investigation no difference was found between the hematocrits taken at the start and at the end of the experiments. The level of the extraction during continuous infusion corresponds well with values found by others (Early & Friedler, 1965; Gagnon et al., 1971) but lower values have also been reported (Sieberth & Mertz, 1965).

After a single injection of iodohippurate in dogs the decrease of the extraction values with time was considerable even when corrected for the slight fall in the concomitantly measured extraction during continuous infusion. In view of the widespread use of single injection of iodohippurate it is remarkable that this decrease of the extraction values, although noted before (Dürr, Nieth, Schollmeyer, Stein & Feine, 1965; Jago, 1971), has received very little attention.

The clearance values after single injection of iodohippurate also showed a marked decrease, corresponding to the decrease in the extraction values.

The investigations in patients showed iodohippurate to behave in a similar way in man as in the dog. There was a marked fall in the extraction after a single injection, but with continuous infusion the extraction was constant. Although the group of patients was rather heterogenous a mean value was calculated in order to facilitate comparison with other studies. The values for the extraction during continuous infusion was within the same range as reported by others (Cheville, Hancock, Davis, Dowdy & Luetscher, 1966; Dabaj, Menges & Pritchard, 1966; Maher, Strong & Elveback, 1971; Vorburger, Gurtner & Reubi, 1972; Lingårdh, 1972).

As mentioned, the decrease of the extraction values after single injection of iodohippurate was marked even after correction for the slight fall in the extraction during continuous infusion. Several explanations may be considered. Even if the iodohippurate used in the single injection experiments contained very little free iodine (0.7 %), this must be assumed to

have some effect, since the relative content of free iodine in plasma increases continuously due to its lower clearance.

If protein-binding of the test substance were of importance the addition of carrier ought to raise the extraction. The effect of carrier, however, was the opposite, the levels of extraction being lower in tests with carrier than in those without. There is thus no reason to assume that protein-binding is a factor of importance for the decrease of the extraction values.

The renal venous blood samples were obtained at exactly the same time as the arterial samples and no correction for renal blood transit time was applied. Calculation on the values obtained in dogs shows that a correction for a mean renal transit time of 10 seconds (Lewis, Bergentz & Leandroer, 1969) means at the most a two per cent higher extraction at one minute after the single injection while no influence of the transit time can be demonstrated after 6 minutes.

Uptake of iodohippurate into the red blood cells does occur and a shift from cells to plasma during the passage of the kidney, as described for iodopyracet by White (1940) and for iodohippurate by Wedeen & Weiner (1969), or during separation of the plasma, could explain the decrease in extraction. Since the calculated, theoretical extraction values were about two per cent higher than the measured ones it can be concluded that a small shift occurs. Considering that the time spent in the kidney is about 10 seconds and that the time from the drawing of a blood sample till the separation of the plasma is 6 - 7 minutes it must be assumed that the shift occurs *in vitro*, as also concluded for para-aminohippuric acid by Phillips, Dole, Hamilton, Emerson, Archibald & Van Slyke (1946) and for iodopyracet by Study & Shipley (1950). Although the calculated extraction values were somewhat higher they decreased with the same rate as the measured ones and red blood cell uptake consequently cannot be offered as an explanation for the decrease in the extraction.

The present investigation was undertaken in order to find out whether the decrease in the clearance values after a single injection of iodohippurate found in a previous investigation (Pihl & Nosslin, 1973) could have been caused by a falling extraction. The decrease rates for the clearance and for the extraction were found to be practically the same. In the previous study, however, the clearance values fell more than can be explained by the decrease in extraction now found, but this discrepancy may be ascribed to the use of venous samples in the earlier study, as shown by Pihl (1973).

Even if the main reasons for the fall in the clearance values have now been revealed it still remains to analyze the mechanism behind the decreasing renal extraction.

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The single injection technique for determination of renal clearance IV.

Clearance calculations and kinetics of the test substances.

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The generally accepted method for determination of renal clearance is the continuous infusion technique, as described by Smith (1951, 1956). Numerous attempts have been made to replace the continuous infusion with a single injection in order to arrive at a simpler and quicker method for clinical use (for survey see Blaufox, 1972). The increasing availability of radioactive test substances has further stimulated such studies. In our laboratory the clearance of radioactive iothalamate and iodohippurate after a single intravenous injection has been analysed (Pihl & Nosslin 1973, Pihl 1973 a, b, c). Several methods have been proposed for the calculation of clearance and distribution volume after single injection, and in this report these methods will be applied to the elimination curves for the above-mentioned test substances. The curves will also be used for an analysis of the kinetics of iothalamate and iodohippurate, with special regard to the possible mechanism for the decreasing renal extraction of iodohippurate earlier reported (Pihl 1973 b).

MATERIAL

The investigations were carried out in dogs and in patients with ^{125}I -iothalamate and ^{125}I -iodohippurate given as single doses without carrier. Usually simultaneous continuous infusion was given of the same substance, labelled with ^{131}I . Arterial blood samples, in some cases also venous samples, were taken during 130 minutes, and urine was collected in six periods of twenty minutes each. A theoretical initial plasma value was calculated from the dose and the estimated plasma volume, and the activity in all subsequent samples was expressed as a fraction of the initial value. Excreted activity was also recalculated to fraction of dose. The curves used for analysis in this report represent mean values from 20 dogs and 10 patients in the case of iothalamate and 19 dogs and 9 patients in the case of iodohippurate. The content of free iodine in the test substances was checked with paper chromatography. No free iodine could be demonstrated in iothalamate, while the iodohippurate preparations used for single injection contained on an average 0.7 % (range 0.3 - 0.9 %) iodine. Further details are given in Pihl (1973 c).

METHODS

Each plasma curve was plotted on semilogarithmic paper and resolved into exponential functions according to the standard 'peeling off' technique, starting from the final straight part of the curve. This slope was defined by the values at 110 and 130 minutes. Each of the four curves was found to require four exponential functions for complete description. Each curve was thus described by four slopes and four intercepts, and all calculations of plasma values, areas and slopes at different times or for different time intervals were performed on a desk computer (Olivetti P 602) with the use of these constants. Clearance was calculated according to methods I, II, III and VI (Table I) and distribution volume according to methods I, II, III, VI, VII and VIII. Most calculation methods demand that the curves have been followed until they have reached their true final slope, and the comparison of the methods was therefore based primarily on values obtained from the complete curves. In order to illustrate the effect of using incomplete plasma curves, i.e. curves from experiments which have been too short to allow the elimination curve to exhibit its final slope, the calculation formulas were also applied to the four curves after they had been deliberately and progressively curtailed. In the latter case the slope of the tangent at the end of the curve was taken as its final slope. Method II (slope method) was also applied to pairs of values on the plasma curve; these plasma values were taken at times often used in the practical application of this method according to findings on perusal of the extensive literature on clinical determination of renal clearance. The same principle was applied in the selection of the four plasma values necessary for an analysis according to method III (Sapirstein method). All clearance and distribution volume values were expressed as percentage of the correct value, which in the case of clearance was taken as the result of the concomitant continuous infusion study. For distribution volume the correct value was defined as the result of method VI, using areas to infinite time.

Table I is a survey of available methods for calculation of clearance and distribution volume after single injection of a test substance. It should be pointed out that the mathematical analysis of the elimination curves is exactly the same whether the process under study concerns renal clearance, metabolic turnover or blood circulation. This unified view of the situation has evolved during recent years and explains why several of the works given as references in Table I primarily deal with kinetic problems other than renal clearance. It is now generally agreed that method I, and the corresponding method VI, represent the most general and correct way of calculation. All other techniques are to be looked upon as approximation methods, giving results that deviate more or less systematically

Table I. Methods for calculation of clearance (C) and distribution volume (V) after single injection.

No.	Method	Parameter calculated	Reference
<u>Total clearance</u>			
I	Area under plasma curve (Stewart-Hamilton principle)	C, V	Zierler (1963) Nosslin (1964, 1965) Matthews (1965)
II	One-pool model (slope method) a. Pool size by extrapolation b. Pool size by other methods	C, V C	Newman, Bordley & Winternitz (1944) Tacket & Houck (1950) Robson (1952)
III	Two-pool model	C, V	Sapirstein, Vidt, Mandel & Hanusek (1955)
IV	Three-pool model	C, V	Matthews (1957) Bianchi (1961)
V	Indirect methods (Correlation with slope, half-time or retention)	-	Barnett (1940) Dost (1949) Wittkopf (1951) Alestig, Hood & Vilgren (1966) Tauxe, Maher & Taylor (1971)
<u>Renal clearance</u>			
VI	Area under plasma and excretion curve	C, V	Bergner (1964, 1965) Nosslin (1964, 1966) Matthews (1965)
VII	Classical clearance formula (UV/P) and dilution principle ($[Dose - Excr.] / P$)	C, V	Alving & Miller (1940) Hogeman (1948)
VIII	Pool from slope and clearance	V	Riggs (1963, p. 212)

from the proper value depending upon the degree of simplification of the theoretical model.

RESULTS AND DISCUSSION

The mean plasma and excretion curves are presented in Fig 1. It can be seen that the two test substances behave rather differently inasmuch as the iodohippurate plasma curve falls more rapidly than the iothalamate curve. There is a corresponding difference in the height of the excretion curve. After two hours plasma contains 3 % of iothalamate in the dog and 8 % in man, while the corresponding figures for iodohippurate are only 0.5 % and 0.4 %, respectively. At the same time 60 - 65 % of iothalamate has been excreted, while 80 - 90 % of iodohippurate has been eliminated.

Calculation of clearance and distribution volume. The main results are presented in Fig 2 and 3 and Table II. The results with iodohippurate seem to be more complex than with iothalamate, for reasons to be discussed later. Comparison of the properties of the different calculation methods is therefore best performed on the iothalamate values.

The renal single injection method (method VI) gives correct results in both dog and man. The calculated total plasma clearance values, on the other hand, are all more or less elevated. The result with method I in man is still within acceptable limits for practical work, while other methods give an appreciable overestimation, especially in the dog. When the total clearance is calculated from incomplete plasma curves increasingly higher values are obtained when the time of the study is shortened. In all cases the slope method (method II) gives the most erroneous result.

The methods for calculation of distribution volume can be roughly divided into two groups according to the results for both iothalamate and iodohippurate. Methods II and VII always give values that are too high while methods I, III and VIII give lower values which are of comparable size and which in the case of iothalamate studies on man all are reasonably correct. In the dog, however, these methods underestimate the distribution volume. When incomplete curves are used for volume estimation, the general tendency is to procure values that are continuously decreasing when the sampling time is shortened. This is the opposite tendency compared to the deviations in the clearance values obtained from incomplete curves.

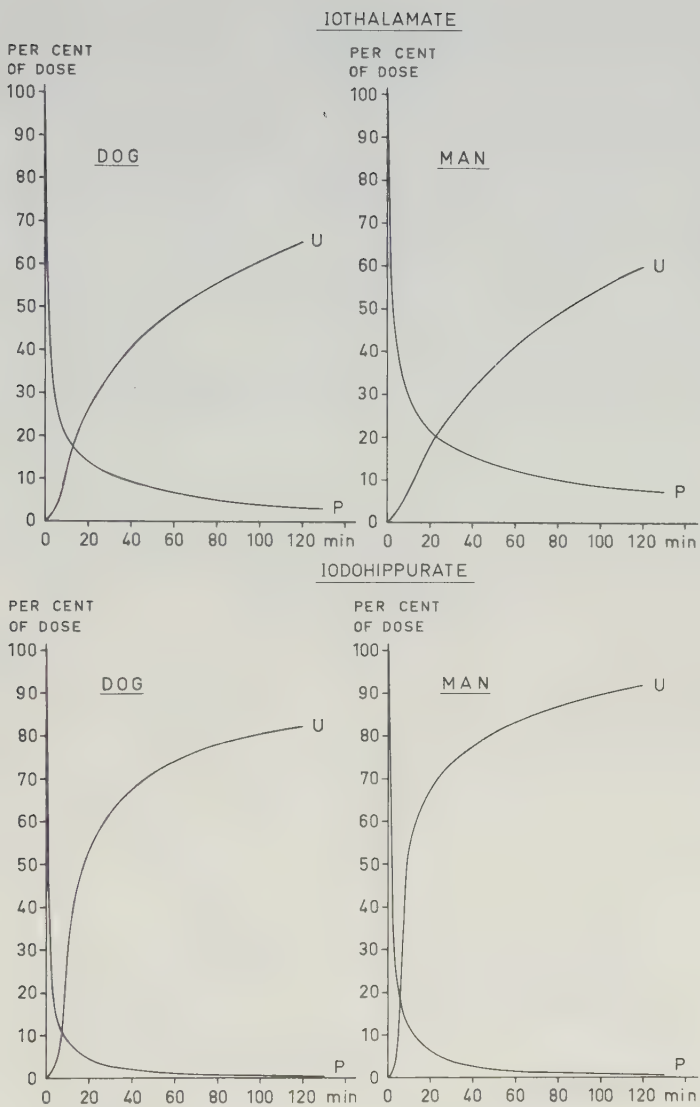


Fig 1. Plasma disappearance curve (P) and urinary excretion curve (U) for iothalamate and iodohippurate in dog and in man.

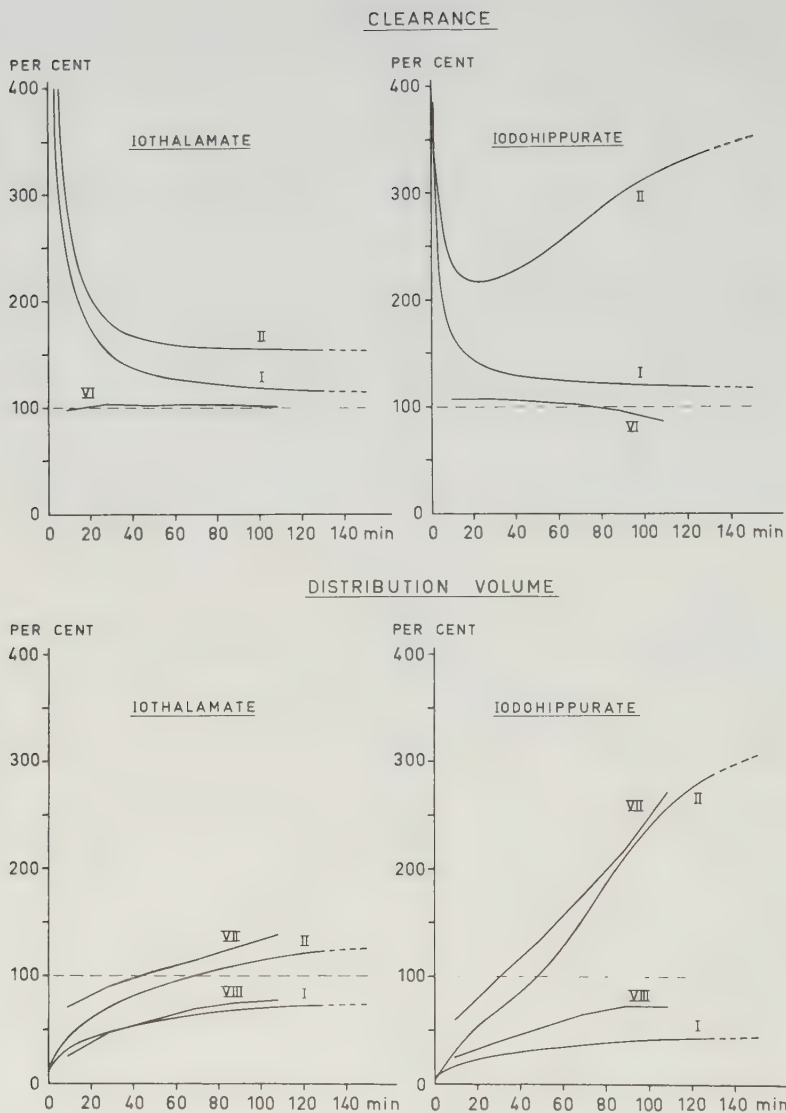


Fig 2. Clearance and distribution volume in the dog calculated with different methods from experiments of increasing duration. Method-numbers according to Table I. All results expressed as percentage of correct value (see text), denoted by dashed line at 100 %.

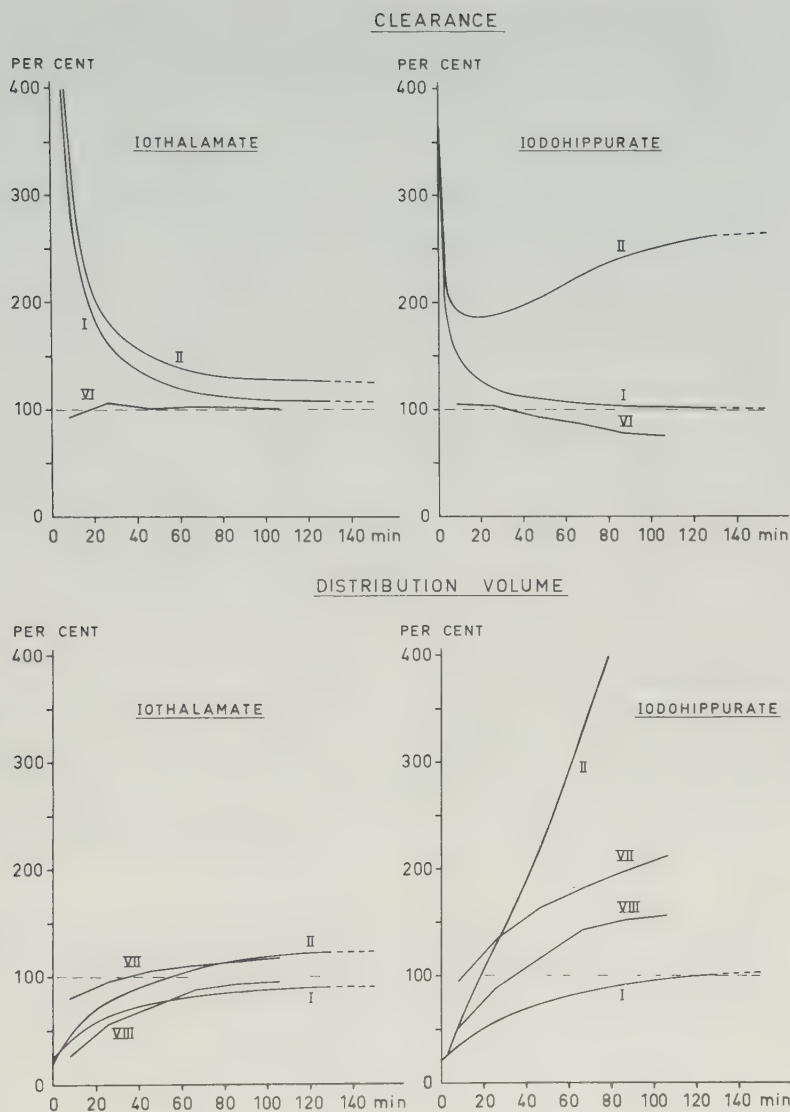


Fig 3. Clearance and distribution volume in man calculated with different methods from experiments of increasing duration. Method-numbers according to Table I. All results expressed as percentage of correct value (see text), denoted by dashed line at 100 %.

Table II. Calculation of clearance (C) and distribution volume (V) with slope method and Sapirstein method from different parts of the plasma curve. Results expressed as percentage of correct value.

Sampling time (min.)	Iothalamate				Iodohippurate			
	Dog		Man		Dog		Man	
	C	V	C	V	C	V	C	V
<u>Slope method (II)</u>								
7 - 17	248	44	278	52	215	35	176	62
10 - 20	227	51	242	59	214	42	179	77
20 - 30	187	66	186	75	216	62	186	123
40 - 60	160	86	145	95	235	101	203	224
60 - 120	151	106	128	113	276	188	229	404
180 - 240	158	128	127	125	356	319	270	586
<u>Sapirstein method (III)</u>								
10-20-30-40	152	60	151	71	164	45	130	80
20-40-60-80	134	77	121	91	167	68	137	141
40-80-120-160	128	87	112	99	163	84	136	177
60-120-180-240	129	89	113	100	172	93	140	188

The findings with iodohippurate are similar to those of iothalamate, but the renal clearance values are continuously falling and the total clearance values show a greater deviation from the true value. The estimates of the distribution volume of iodohippurate also differ more from the correct value. The curves found on calculations with incompletely followed curves also display this phenomenon and it can be seen that the increasing clearance values obtained with method II at later times depend mainly on the extreme overestimation of the distribution volume with the slope method. This may in some way be connected with the cellular uptake of iodohippurate. When the curves are continuously shortened, the same tendency is demonstrated in the iodohippurate distribution volumes as in the iothalamate volumes, i.e. all values decrease.

The available methods can thus give systematically different results with a general tendency to overestimation of the clearance and underestimation of the distribution volume. In the case of iothalamate the use of long curves is advisable while such curves may be dangerous in the case of iodohippurate because of its much faster elimination from plasma. The practical conclusion must then be that when the clearance is to be determined with single injection technique urine sampling is necessary if short-term determination is desired, and even then iodohippurate gives results that may be difficult to interpret clinically. When total plasma clearance is determined without urine sampling it is important that the complete elimination process is followed in plasma. From this point of view a two-hour curve is sufficient in the case of iodohippurate, while a longer observation time is necessary for correct calculation of the total clearance of iothalamate.

Influence of free radioiodine on clearance values. The iodohippurate solution used in these experiments had a mean content of 0.7 % free iodine. Higher contents, up to 5 %, may be found in ^{131}I -labelled iodohippurate. There is no general agreement in the literature concerning the degree of influence, although it is admitted that the free iodine must lower the clearance values (see Pihl, 1973 a). Direct studies of the iodine content in plasma or urine is difficult since large volumes and complicated extraction procedures would be necessary in order to measure the content. An alternative way of analysing the situation is presented in Table III, where published plasma values for radioiodine after single injection have been used for calculation of its relative concentration at different times after injection of an iodohippurate solution. It is seen that although the initial iodine content is only 0.7 % of the injected activity, already at two hours the iodine constitutes more than ten per cent of the plasma activity. As a consequence the clearance and the extraction at

Table III. Influence of free iodine in iodohippurate on plasma concentration, clearance and extraction values

Time min.	Total plasma activity	Iodine activity			Iodo- hippurate activity	Resulting clearance		Resulting extraction
	% x 10 ³	%/liter	% x 10 ³	% of total	% x 10 ³	ml/min	%	%
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
0	100000	32.3	700	0.7	99300	596	100	92
10	11735	5.7	124	1.1	11611	594	100	92
30	3853	4.5	98	2.5	3755	586	98	90
50	2084	3.8	83	4.0	2001	577	97	89
70	1335	3.5	76	5.7	1259	568	95	88
90	912	3.3	71	7.9	841	555	93	86
110	688	3.0	65	9.4	623	546	92	84
130	536	2.8	61	11.4	475	535	90	83

Comments:

- Column (2) Mean plasma curve in man after injection of ¹²⁵I-iodohippurate; initial value calculated from dose and estimated plasma volume and taken as 100 per cent in each case.
- Column (3) Radioiodine plasma curve in normal man, from Hays & Wegner (1965) Fig 2. Initial value calculated from the given plasma volume value of 3100 ml.
- Column (4) Initial value corresponds to a free iodine content of 0.7 %. All later values calculated by application of the values for the iodine plasma curve in column (3).
- Column (5) Relative iodine content in plasma, obtained by expressing values in column (4) as a percentage of total activity (column 2).
- Column (6) Obtained by subtraction of values in column (4) from values in column (2).
- Column (7) Calculated with use of iodine percentage in column (5) and assuming an iodohippurate clearance of 600 ml/min and an iodine clearance of 30 ml/min.
- Column (9) Extraction rate for pure iodohippurate assumed to be 92 %.

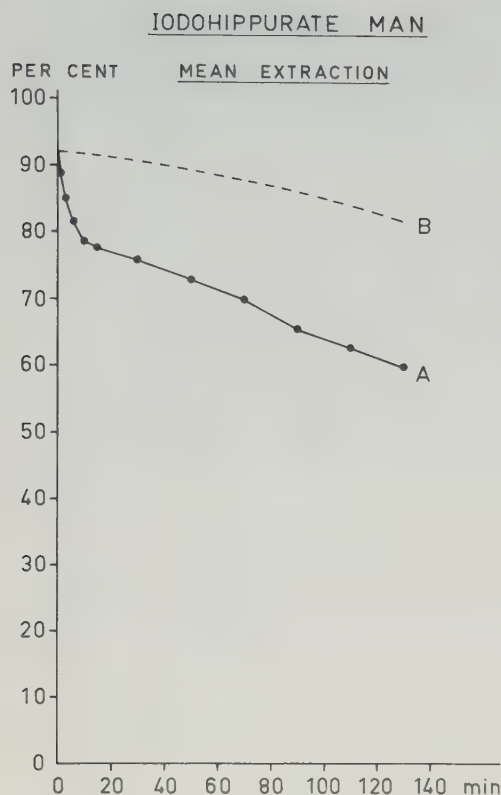


Fig 4. Renal extraction of iodohippurate after single injection (A). Mean values from 9 patients studied by Pihl (1973 b). Curve B denotes theoretical extraction values for iodohippurate containing 0.7 % free iodine, assuming a constant extraction of 92 % for pure iodohippurate (Table III, column 9).

this moment has been reduced by ten per cent. This marked effect is due to the great difference between the normal clearance of iodohippurate and that of iodine. When the same type of calculation is performed assuming an initial content of 2 % free iodine, the corresponding decrease is 35 %. If, however, the calculation of clearance is based on the total interval from the time of injection to two hours, a mean value will be obtained which is much less disturbed by the iodine content. This is because most of the iodohippurate is eliminated in the beginning of the period, when the iodine content is still rather low. It is thus important to distinguish between two types of estimations in this respect, one utilizing information from the whole interval up to a certain time, the other using only the measured values at a specified moment; the disturbing influence of iodine is maximal in the latter case. The phenomenon is well illustrated by the finding of a change of only 1.2 % on recalculation of the total plasma clearance of iodohippurate in man after correction for iodine according to Table III. However, in the experiments performed (Pihl 1973 b, c) the clearance values decreased by as much

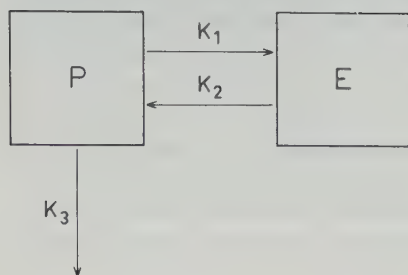
as 19 % and the extraction values by 32 % in two hours in man; this pronounced fall can only partly be explained by the presence of 0.7 % free iodine in the test substance (Fig 4). Therefore, it seems necessary to assume a kinetic mechanism in the excretion of iodohippurate which also leads to a diminishing extraction.

Kinetics of iothalamate and iodohippurate. On the basis of certain general properties of the elimination curves such as area, center of gravity and initial slope it is possible to define adequate kinetic models for the test substances. The following discussion pertains to the condition in man, but the same principles can be used for delineating models in the dog. The figures given for rates and pool sizes in Figs 5 and 6 should not be looked upon as unique and exact solutions of the kinetic formulas but more as reasonable values illustrating the relative sizes of the flows and pools.

In the case of iothalamate a simple two-pool model is sufficient for description of the main processes, namely the distribution into extravascular parts of the extracellular space and the renal clearance at a constant extraction rate. The values in Fig 5 have been obtained in the following way: K_3 is equal to the fractional clearance, i.e. the quotient between clearance and plasma volume. K_1 is the difference between the initial slope in the plasma curve (found to be 0.35 parts per minute) and K_3 . The sum of the plasma volume and the extravascular space must be equal to the calculated total distribution volume. Finally K_2 is calculated as the quotient between K_1 and the size of the extravascular space. A similar model with similar values for the pools and rate constants can be expected to be valid for other substances used in measurements of glomerular filtration rate, such as Cr-EDTA and diatrizoate. If the distribution volume is expressed as percentage of body-weight a mean value of 18.4 % is obtained in man, which is in good agreement with the size of the extracellular space.

The kinetics of iodohippurate seems to be more complicated, since the decrease in clearance after single injection makes the assumption of a further, renal compartment necessary. However, in order to arrive at approximate figures for the exchange rates between plasma and all other extrarenal spaces, it is suitable first to analyse the values with the same method as used above for iothalamate. The value given for K_2 in Fig 6 has been so obtained, using a fractional clearance value of 0.17 and an initial slope of 0.60 parts per minute, together with a distribution volume 5.4 times the plasma volume. For K_3 , which stands for the glomerular filtration process, the value 0.03 is taken from the iothalamate model.

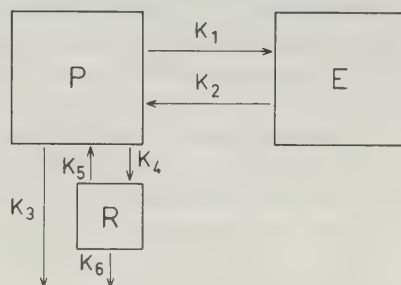
IOTHALAMATE



	DOG	MAN
K_1	0.42	0.32
K_2	0.07	0.10
K_3	0.056	0.031
P	1.0	1.0
E	6.0	3.2
DISTR. VOLUME	7.0	4.2
CLEARANCE	0.056	0.031

Fig 5. Kinetic model for iothalamate, consisting of a plasma pool (P) and extravascular pools (E). Rate constants for flows denoted by K (min^{-1}). Clearance and distribution volume given as fractions of the reference pool P.

IODOHIPPURATE



	DOG		MAN	
EXTR. %	75	87	80	92
K_1	0.457	0.491	0.39	0.42
K_2	0.037	0.037	0.10	0.10
K_3	0.056	0.056	0.03	0.03
K_4	0.187	0.153	0.18	0.15
K_5	0.037	0.037	0.08	0.06
K_6	0.077	0.170	0.28	0.56
P	1.0	1.0	1.0	1.0
E	12.4	13.3	3.9	4.15
R	1.6	0.7	0.5	0.25
DISTR. VOLUME	14.0	14.0	5.4	5.4
CLEARANCE	0.182	0.182	0.17	0.17
K_5/K_6	33/67	18/82	22/88	10/90

Fig 6. Kinetic model for iodohippurate, consisting of a plasma pool (P), extravascular pools (E) and a renal pool (R). Rate constants for flows denoted by K (min^{-1}). Clearance and distribution volume given as fractions of the reference pool P.

The remaining constants have been calculated assuming two different values for the renal extraction of pure iodohippurate during continuous infusion. When a steady state has been attained, all flows into a pool must equal the flows out of the pool. This condition defines the necessary equations for solving for K_1 , K_4 , K_5 and K_6 . Finally the size of the renal compartment can be calculated from the equation $R = K_4 / (K_5 + K_6)$. It is seen that the assumed renal compartment has a size comparable to the plasma pool. In man, depending upon the extraction rate, 10 - 22 % of the amount originally taken up in the kidney is returned to plasma, while in the dog the corresponding calculated values are 18 - 33 %.

A similar kinetic model for iodohippurate has been proposed by Conn, Sabo, Landes & Ho (1964), based on the observation of inconstant clearance values with changing plasma concentrations. They assume that the renal compartment is made up by the interstitial fluid between renal capillaries and tubular cells, and look upon the re-entry as a back-diffusion of test substance. A back-diffusion was also demonstrated by Schnermann & Thureau (1965) in studies on the excretion of para-aminohippuric acid in golden hamsters.

It should be underlined that from a kinetic point of view the extravascular space cannot be expected to behave as a single homogenous pool. This explains why formulas based on simple two- or threepool models are more or less inadequate for calculation of clearance and distribution volume, and also why four exponential functions were found necessary to describe the disappearance curves completely.

Concluding remarks

Several objections have been raised to the use of single injection techniques for determination of renal or total clearance. Most of the criticism can be brought back to the following four main problems, already discussed by Smith (1951).

a) The distribution volume. In the correct general area methods the plasma volume is used as the reference volume, but exact knowledge of this volume is not necessary for the calculations. It does not enter the formulas and is used only to estimate the initial concentration in plasma when the area is measured (Pihl & Nosslin, 1973). All other methods employ so called 'apparent' distribution volumes, calculated in different ways and often showing continuous increase in size during the experiment. This increase is due to the fact that complete equilibration within the total distribution

volume can never be attained in an open biological system of several compartments. Since knowledge of the total distribution volume is not required and since equilibrium is not assumed in the calculation of clearance with the area methods, this problem is now no longer of importance.

b) Final slope in the plasma curve. When simple pool-models are used for calculation, it is essential that the plasma curve has been followed until the final slope has been reached. For some substances, such as inulin, constant slope is not obtained even after several hours (Robson, 1952). In correct methods it is still theoretically necessary to know the final slope for calculation of the total area, but the error introduced when incomplete curves are analysed is much less if the total area under the curve is used instead of only values from the later part of the curves. When renal clearance is determined, no restrictions need to be put on the shape of the plasma curve.

c) The arterio-venous difference. This is still an obvious source of error, especially in the case of substances having a high normal clearance, such as iodohippurate. The influence is smaller for substances eliminated by glomerular filtration, and in general, for each substance, the error decreases with decreasing clearance. When falling plasma values are used, the venous clearance values are usually too low. None the less venous blood samples are often used in clinical practice.

d) Delay correction. When urine sampling is used, correction must be applied for the delay in the urinary tract, which functions as a dead space. If the diuresis is good and if there are no gross changes in the urinary tract, the delay correction does not seem to be difficult, and its influence can be greatly diminished by the use of long collection periods (Pihl, 1973 a).

In conclusion, simple and correct calculation methods are now available for single injection clearance determinations, and the part of the criticism which has emanated from the application of erroneous formulas can be disregarded. For some substances, however, other practical problems may still make single injection methods difficult to use in routine. One such problem is the content of free iodine in iodine-labelled compounds.

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The single injection technique for determination of renal clearance V.
A comparison with the continuous infusion technique in the dog and in man.

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After single injection of iodohippurate in the dog a pronounced fall in the clearance values from period to period was found by Pihl & Nosslin (1973). In further investigations several factors were studied in search of an explanation for this fall (Pihl, 1973 a, b; Nosslin & Pihl, 1973). It was found that the decrease in the clearance values was chiefly due to two factors, the first of which, namely the difference in concentration of the test substance between arterial and venous blood could easily be avoided. The second factor was a decreasing renal extraction, to some extent caused by the presence of free iodine in the test substance but in the main due to the specific renal handling of iodohippurate. With this knowledge at hand it was decided to reinvestigate the clearance of iodohippurate as well as of iothalamate in the dog and in man.

MATERIAL AND METHODS

Animals. Twelve female, mongrel dogs weighing 13 - 25 kgs were used. They were kept on the ordinary diet of the laboratory. During at least twelve hours before each experiment they were given no food but allowed water ad libitum.

Patients. Twentythree patients from the Department of Urology underwent clearance determinations. In no case was the kidney function severely reduced. Serum creatinine was within 0.8 - 1.6 mg/100 ml. The patients were of both sexes and varied in age between 21 and 79 years.

Test substances. Sodium iothalamate and sodium ortho-iodohippurate labelled with ^{125}I or ^{131}I were purchased from the Radiochemical Centre, Amersham, England. After appropriate dilution they were kept in the dark in the refrigerator. Substances labelled with ^{131}I were never kept for more than two weeks. Each batch of the test substances was checked for free iodine as earlier described (Pihl, 1973 a). The content of free iodine was in all cases below 1 % for ^{125}I and with few exceptions below 2 % for ^{131}I . For every experiment 50 - 100 μCi were used, corresponding to 0.5 - 20 mg of iothalamate, and 0.25 - 10 mg of iodohippurate. The single injection dose, the priming dose and the dose standard were made up as earlier described (Pihl & Nosslin, 1973).

Experimental procedure. The clearance determinations in dogs were carried out under general anesthesia with endotracheal intubation. The ventilation was maintained with a constant volume respirator. In Series I sodium pentobarbital was used and in Series II the anesthesia was induced with sodium thiopental and maintained with a mixture of N_2O and O_2 and suxamethonium chloride as a muscle relaxant. After tying the dogs in a supine position on an animal board a bladder catheter was applied, the priming dose was given and the continuous infusion, containing 5 % mannitol, was started. About two hours later the bladder was emptied and the single injection was given. At 1, 3, 6, 10, 15, 30, 50, 70, 90, 110 and 130 minutes blood samples were drawn from a plastic catheter previously placed in the femoral artery. Centrifugation was performed immediately. The total blood loss for the animal during one experiment was about 130 ml corresponding to a mean plasma 'clearance' of 0.04 ml/min/kg. Six urine collection periods of 20 minutes each were run. At the end of the experiment 1 ml aliquots of all blood, plasma and urine samples were taken by constriction pipettes into special plastic tubes for counting. Details regarding the experimental procedure can be found in Pihl (1973 b).

For clearance determinations in patients the procedure was in principle as for the animals and only differences are described. The patients were allowed a light breakfast and were given about 500 ml of water orally in the morning. They were kept in bed. Most patients had an indwelling Foley catheter, which was replaced by a sixhole catheter. The single injection, the priming dose and the continuous infusion were given through an indwelling intravenous cannula in an armvein. The continuous infusion fluid was 5 % glucose and the rate of administration 5.4 ml per minute. Blood samples were obtained from plastic catheters, placed by the Seldinger technique under local anesthesia in the femoral artery and vein. Six clearance periods were run, the bladder being flushed with 2 x 30 ml of saline and repeatedly with air between each period.

Series. Two series of dogs were run. Due to various technical reasons seven experiments had to be discarded. Series I consisted of 20 experiments, where ^{125}I -iothalamate was given as a single injection and ^{131}I -iothalamate as a continuous infusion. In Series II were 19 experiments, in which ^{125}I -iodohippurate was given as a single injection. In ten of these ^{131}I -iodohippurate was given as a continuous infusion. In the remainder various other test substances were used either as single injections or as continuous infusions (results not presented here).

Two series of patients were investigated. Four investigations were excluded, three because of technical errors and one because of too low

urine production. In the first series were 10 patients receiving ^{125}I -iothalamate as a single injection and ^{131}I -iothalamate as a continuous infusion. Venous as well as arterial blood was obtained in nine cases. The second series consisted of 9 patients who were given ^{125}I -iodohippurate as a single injection and ^{131}I -iodohippurate as a continuous infusion. In eight cases arterial and venous blood was obtained.

Measurement of radioactivity. The radioactivity was measured in 1 ml samples of plasma or blood and urine in a Packard automatic gamma spectrometer model 5212 using standard dual channel technique as earlier described (Pihl & Nosslin, 1973). A fixed counting time of ten minutes was used. The counting error as calculated according to formulas given by Ambrosen (1967) were for iothalamate whether given as a single injection or as a continuous infusion below 2.0 %. For iodohippurate the error was below 5 % irrespective of mode of administration.

Calculations. The continuous infusion clearance was calculated according to the standard formula:

$$C = \frac{U \times V}{P}$$

where U = cpm per ml urine, P = cpm per ml plasma or blood and V = urine flow in ml per minute.

The single injection clearance was calculated according to the formula:

$$C = \frac{E}{A}$$

where E = total amount excreted in urine during a certain time, and A = area under the plasma curve during the corresponding time period (Pihl & Nosslin, 1973). In the dog experiments a delay time correction of two minutes was used (Pihl, 1973 a). For patients the delay time was calculated according to a formula given by Nosslin (1965):

$$T = 3.6 + 2.6/V$$

where T = delay time in minutes and V = urine flow in ml per minute. Usually the delay amounted to 4 minutes. A clearance value was calculated not only for each 20 minute period but also for the total time 0 - 120 minutes. This value is consequently not identical with the mean value of the six clearance periods.

Statistical methods. Statistical comparisons were based on Student's t-test for paired observations and on analysis of variance for groups of observations. Regression within groups was studied with analysis of covariance, otherwise standard statistical procedures were used (Snedecor, 1955). Regression expressed as per cent change per period was obtained by multiplying the regression coefficient by period length x 100 and dividing by the mean clearance value. Differences found were considered probably significant if $P < 0.05$ and significant if $P < 0.01$.

RESULTS

Iothalamate. The plasma levels of iothalamate during continuous infusion clearance measurements in the dog were practically constant although a statistically significant increase of 0.6 % per period was found. In man the levels were constant.

The main results of the clearance measurements in the dog as well as in man are given in Tables I and II and in Fig 1. Since there were no significant differences between plasma and blood, the former values only are presented.

In the dog the single injection clearance showed an excellent agreement with the continuous infusion clearance. There was no significant difference between the mean values and the correlation coefficient was 0.97 (Fig 3). Both techniques showed the same slight increase in clearance values during the experiment, and both had an error of the method of about 5 %. The total clearance was 17 % higher than the continuous infusion clearance. The correlation between them was good (Fig 3).

In man the continuous infusion clearance was constant, and although the arterial single injection clearance showed a small increase there was no significant difference between the paired mean values and the correlation coefficient was 0.99 (Fig 4). The venous single injection clearance showed a decrease of 1.6 % per period but there was no significant difference between the arterial and the venous mean single injection clearance values. The error of the method was about the same for all techniques. The total plasma clearance was 5 - 7 % higher than the continuous infusion clearance; there was a high degree of correlation between them (Fig 4).

IOTHALAMATE CLEARANCE

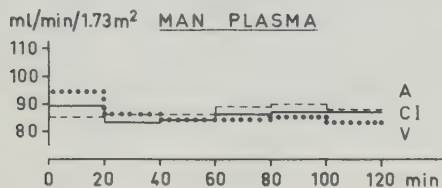
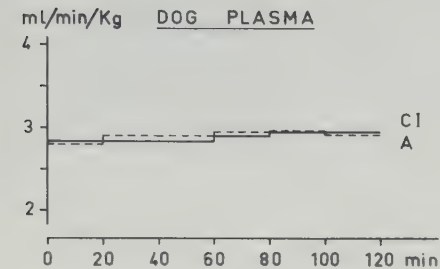


Fig 1. Clearance of iothalamate after single injection and during continuous infusion in dogs and in man. CI=continuous infusion, A=arterial samples, V=venous samples.

Fig 2. Clearance of iodohippurate in plasma and blood after single injection and during continuous infusion in dogs and in man. CI=continuous infusion, A=arterial samples, V=venous samples.

IDOHIPPURATE CLEARANCE

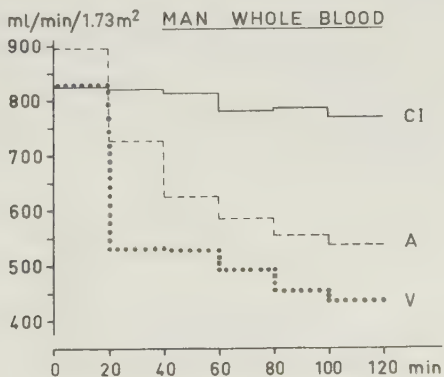
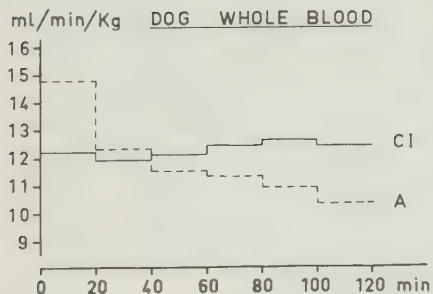
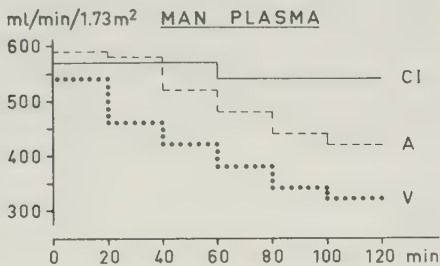
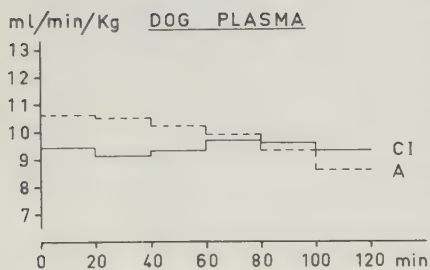


Table I. Results of clearance measurements in dogs

Test substance and Method	Number of expe- riments	Clearance periods						Mean clearance		Total clearance		Regression ^a change/period	Error of method ^b
		I	II	III	IV	V	VI	m	SD	m	SD		
		ml/min/kg										%	
<u>Iothalamate</u>													
Single injection, plasma	20	2.80	2.89	2.88	2.94	2.96	2.93	2.86 ± 0.44		3.39 ± 0.51		+ 0.9**	5.6
Continuous infusion, plasma	20	2.83	2.83	2.84	2.91	2.96	2.95	2.89 ± 0.44				+ 0.9**	5.2
<u>Iodohippurate</u>													
Single injection, plasma	19	10.58	10.46	10.15	9.93	9.32	8.62	10.41 ± 1.88		12.10 ± 2.11		- 3.9**	8.5
Single injection, blood	19	14.80	12.28	11.49	11.33	10.91	10.31	13.56 ± 2.43		15.64 ± 2.65		- 5.4**	7.1
Continuous infusion, plasma	10	9.40	9.08	9.29	9.66	9.55	9.33	9.38 ± 2.32				+ 0.4	5.6
Continuous infusion, blood	10	12.19	11.91	12.07	12.44	12.56	12.43	12.27 ± 2.95				+ 0.8*	5.3

^a Significance denoted by - for P > 0.05, * for P < 0.05 and ** for P < 0.01^b Coefficient of variation for an single period clearance value.

Table II. Results of clearance measurements in man

Test substance and Method	Number of expe- riments	Clearance periods						Mean clearance		Total clearance		Regression ^a		Error of method ^b
		I	II	III	IV	V	VI	m	range	m	range	change/period	%	
<u>Iothalamate</u>														
Single injection, arterial samples	10	93	96	96	99	100	98	96	74 - 146	103	85 - 153	+ 1.1*	7.5	
Single injection, venous samples	9	104	96	94	94	95	93	97	76 - 142	101	75 - 152	- 1.6*	6.9	
Continuous infusion	10	99	93	94	96	97	97	96	72 - 149			- 0.0 ⁻	6.3	
<u>Iodohippurate</u>														
Single injection, arterial samples, plasma	9	585	584	524	480	440	420	568	340 - 863	561	346 - 828	- 6.5**	14.5	
Single injection, arterial samples, blood	9	895	727	624	586	554	537	801	455 - 1202	793	451 - 1156	- 8.4**	15.0	
Single injection, venous samples, plasma	8	536	463	418	384	343	324	490	314 - 725	483	314 - 711	- 8.5**	14.6	
Single injection, venous samples, blood	8	827	531	528	491	453	436	710	431 - 1050	703	429 - 1059	- 10.0**	14.5	
Continuous infusion, plasma	9	570	572	573	539	537	536	557	335 - 862			- 1.6**	7.2	
Continuous infusion, blood	9	825	819	813	779	784	768	798	433 - 1249			- 1.5**	6.4	

^a Significance denoted by - for P > 0.05, * for P < 0.05 and ** for P < 0.01^b Coefficient of variation for a single period clearance value.

IOTHALAMATE CLEARANCE DOG

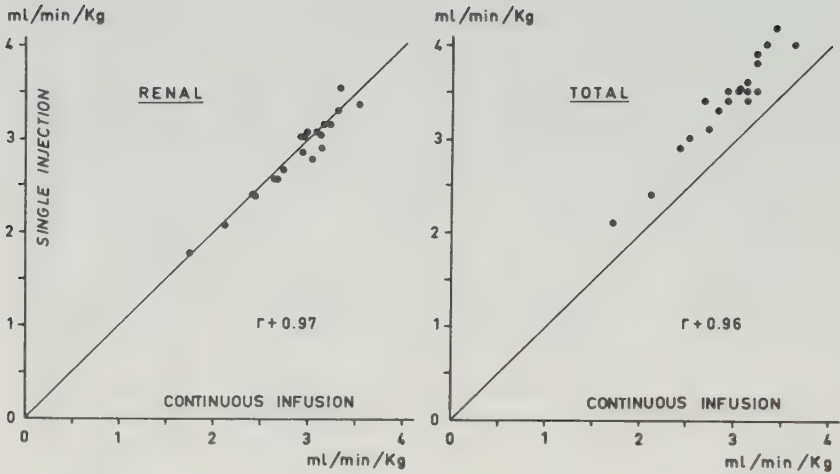


Fig 3. Correlation between arterial single injection clearance, arterial total plasma clearance and continuous infusion clearance of iothalamate in dogs. Line of identity inserted.

IOTHALAMATE CLEARANCE MAN

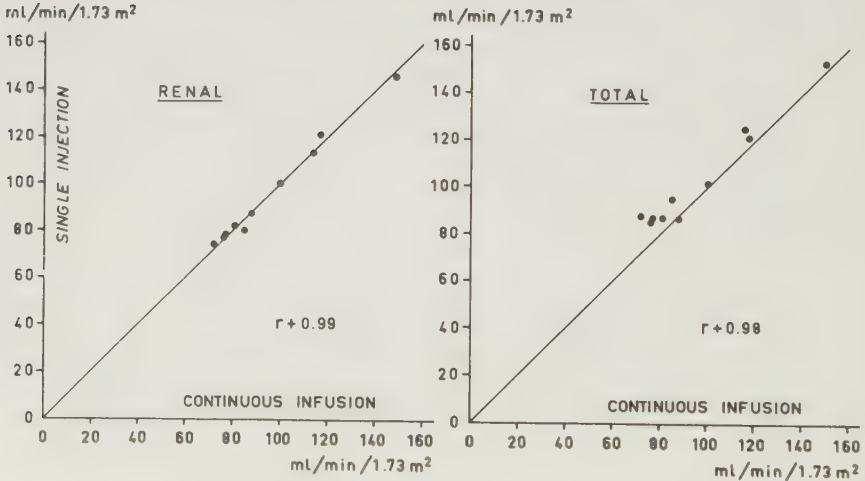


Fig 4. Correlation between arterial single injection clearance, arterial total plasma clearance and continuous infusion clearance of iothalamate in man. Line of identity inserted.

IODOHIPPURATE CLEARANCE DOG

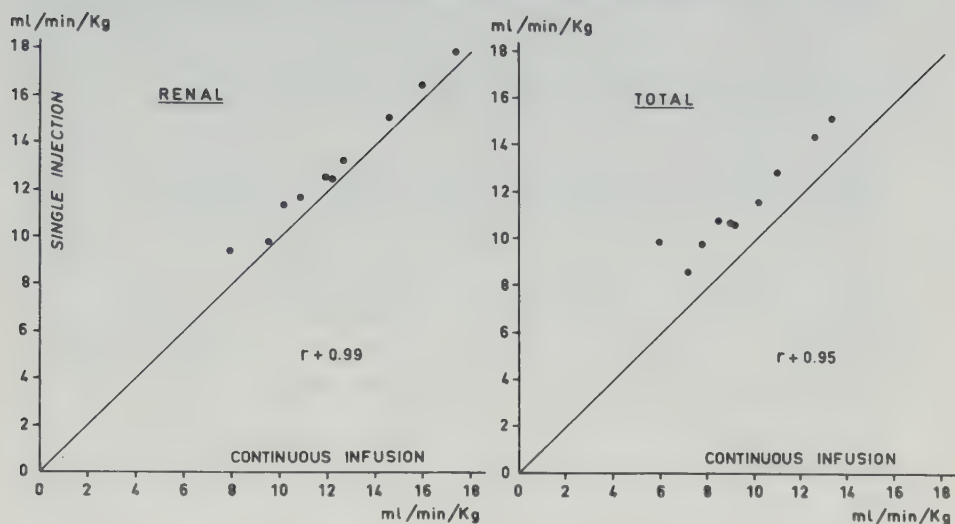


Fig 5. Correlation between arterial single injection clearance, arterial total plasma clearance and continuous infusion clearance of iodohippurate in dogs. Line of identity inserted.

IODOHIPPURATE CLEARANCE MAN

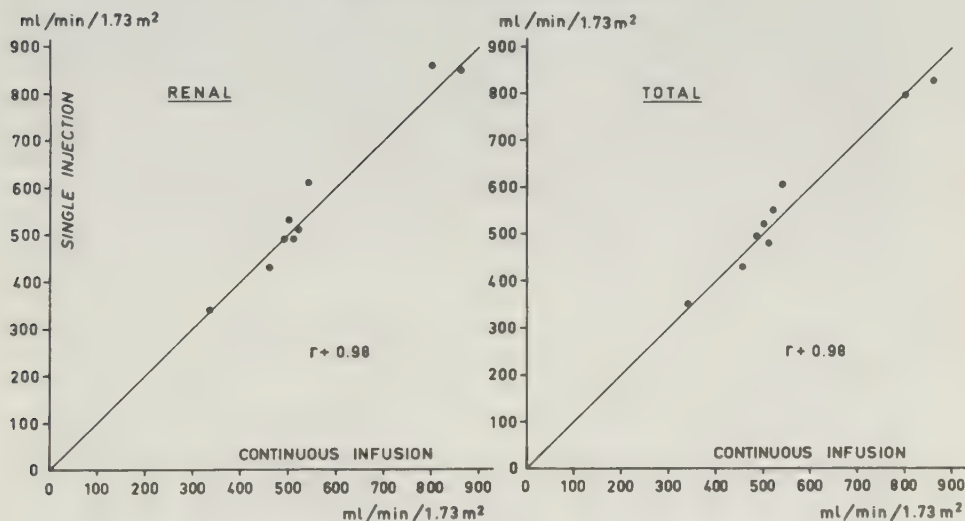


Fig 6. Correlation between arterial single injection clearance, arterial total plasma clearance and continuous infusion clearance of iodohippurate in man. Line of identity inserted.

Iodohippurate. The plasma levels of iodohippurate during continuous infusion in the dog were essentially constant, showing only a small decrease of 0.9 % per period. The maximum deviation from the mean level in each case was 7 %. In man the plasma levels were constant and the deviation from the mean level never exceeded 8.5 %.

The main results of the iodohippurate measurements are assembled in Tables I and II and in Fig 2. Since the changes were always in the same direction in blood and in plasma only the latter values are mentioned.

In the dog the continuous infusion clearance was constant, but the single injection clearance showed a significant decrease amounting to 3.9 % per period. The mean value was 10 % higher than the continuous infusion value, but the correlation between them was excellent (Fig 5). The error of the method was about 8 % for the single injection clearance and 6 % for the continuous infusion clearance. The total plasma clearance was 22 % higher than the continuous infusion clearance in the same animals. The correlation between the total and the continuous infusion clearance was good (Fig 5).

The continuous infusion clearance in man showed a decrease with time of 1.6 % per period while the single injection clearance showed a marked decrease, amounting to 6.5 % per period. There was, however, no significant difference between the paired mean values and there was a high degree of correlation (Fig 6). The venous single injection clearance decreased even more per period and the mean value was 14 % lower than the arterial clearance. The error of the method was about 15 % for single injection and 7 % for continuous infusion. The arterial total plasma clearance was the same as the continuous infusion clearance but the venous clearance was 14 % lower. The correlation between the total plasma clearance and the continuous infusion clearance was good (Fig 6).

DISCUSSION

The animal experiments were performed under general anesthesia, which may depress the renal function (de Bodo & Prescott, 1945; Surtshin, Mueller & White, 1952; Blake, 1957). Also suxamethonium chloride has been found to influence the renal function (Granberg & Wåhlin, 1962; Grängsjö & Persson, 1971). In the present experiments,

however, the animals were their own controls and the influence of the anesthesia was therefore of minor importance.

In the dog a slight increase with time in the continuous infusion clearance values for iothalamate may well have been caused by an initial depressing action of the anesthesia. The single injection clearance increased in an exactly parallel manner and there was no significant difference between them. In man the continuous infusion clearance of iothalamate was constant and although the single injection clearance showed a probably significant increase this was very small. There was no significant difference between the mean values of the two techniques, and the correlation between them was excellent. The venous single injection clearance values showed a significant decrease from period to period, corresponding to the increase in arterio-venous difference earlier demonstrated (Pihl, 1973 a).

For iodohippurate, in contrast to iothalamate, there was a considerable difference between clearance values obtained from plasma and from whole blood. Most of this difference, however, depended on the first clearance period and was due to the uptake of the test substance into the red blood cells. During the later periods the changes were essentially parallel in plasma and in blood. The continuous infusion clearance of iodohippurate was constant in the dog during the test, but the single injection clearance decreased significantly. This fall corresponds closely to the decrease in the extraction rate found in the same animals (Pihl, 1973 b). After adjustment for the slight change with time of the continuous infusion clearance in man the decrease of the single injection clearance is very nearly the same as the decline in the extraction rate earlier demonstrated (Pihl, 1973 b). The decreasing clearance values thus depend on a changing renal extraction, which in its turn is due partly to the presence of free iodine and partly to a re-entry into the blood of substance taken up by the kidney (Nosslin & Pihl, 1973). The difference in the rate of decrease between the arterial and the venous single injection clearances is explained by the increasing arteriovenous difference found in the same patients (Pihl, 1973 a).

In the dog the total plasma clearance of both iothalamate and iodohippurate was higher than the continuous infusion clearance. In man the iothalamate total clearance as calculated on arterial or venous values was somewhat higher than the continuous infusion clearance. For iodohippurate the arterial total clearance was equal to the continuous infusion clearance while the venous total clearance was too low. Since no extrarenal clearance of any magnitude exists for either iothalamate or iodohippurate in the presence of normal kidneys (Griep & Nelp, 1969; Magnusson, 1962), the reason for the differences between the total and the renal clearances is probably to be found in the estimation of the area under the plasma curve.

If the curve has not reached its final slope the total area will be underestimated and the plasma clearance will be too high. This is especially true in the case of iothalamate where 35 - 40 per cent is still retained in the body after two hours (Nosslin & Pihl, 1973). Iodohippurate, on the other hand, is in man almost completely excreted after two hours, fitting well with the finding of a correct value for total plasma clearance. In the dog, probably due to the higher cellular uptake, more iodohippurate is retained after two hours, and this is reflected in the overestimation of the total plasma clearance. However, the iodohippurate plasma curve cannot be followed much more than two hours since the disturbing influence of free iodine increases rapidly at low iodohippurate levels (Nosslin & Pihl, 1973).

For iothalamate it may be concluded that from a practical clinical point of view the renal single injection method in man gives clearance values comparable to those of the continuous infusion method whether arterial or venous blood or plasma is used. A correct total plasma clearance can be obtained from arterial as well as from venous samples if a prolonged (more than two hours) sampling period is applied. For iodohippurate the situation is quite different. Whether arterial or venous blood or plasma samples are used the single injection method does not give clearance values that are comparable to those of the continuous infusion method. The two-hour total plasma clearance, however, is correct if calculated on arterial values.

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